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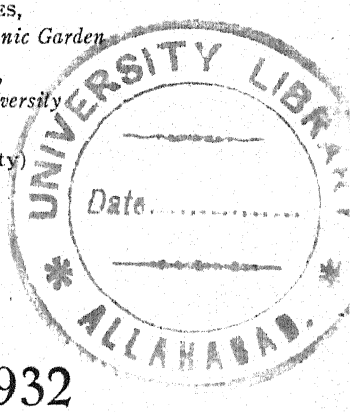

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ALCOHOL SEPARATION OF EMPTY SEED, AND ITS EFFECT ON THE GERMINATION OF RED SPRUCE

HENRY I. BALDWIN

(Received for publication July 11, 1931)

INTRODUCTION

In all experimental work it is important to eliminate variables wherever possible; especially in forestry and ecological experiments in the field, where the number of variables is large and often unknown, any simplification by their removal or control is to be welcomed.

In seed investigations the condition of the seed itself is often an unknown factor. A considerable number of investigations might be mentioned, carried out at great expense, where seeds of various species, frequently a counted number of seed, have been sown under varying conditions, and the germination and survival of the seedlings studied; in many cases the results, viewed critically, cannot be accepted as satisfactory since the quality of the seed at the start was not examined. Uniform material is necessary where only a limited number of duplicate tests is possible. One of the commonest variables in forest tree seed is the percentage of empty or "blind" seed. As the seed comes from the cone in nature this percentage may vary from 30 to 90 depending on the species, size of the seed crop, and degree of adaptation of the mother tree to the site. Where local climatic races have been interchanged in forestry plantings, the percent of empty seed may be excessive and greatly affect the quality of the seed. Through faulty cleaning methods a greater or less proportion of empty seed is to be found in commercial seed obtained from seed extracting plants. In some genera such as *Larix* empty seeds are very difficult to remove mechanically, or detect from inspection of the seed. In *Betula* and similar seeds with thin seed coats, empty seeds and those with undeveloped embryos can be distinguished under low magnification when passed on a glass slide over a strong beam of light, such as in a "diaphanascope."

In any case, the results of germination tests of seed samples containing considerable percentages of unfilled seed should be expressed as percentages

of the number of developed seed present, and capable of germination, which actually germinate. Jacobs (12*a*) proposed the term "real germination percent" to designate this method of calculation; Barton (2) based it on rancid as well as empty seeds, and the percentage was determined from a cutting test on a sample of 100 seeds, similar to the one used in the germination test. This takes account of seed which may be "good" at the start of the test, but which decay in the germinator; however, it is open to the objection that the sample used for the cutting test may vary to an unknown degree from the sample used for the germination test. In the results here reported "real germinations" are based on the number of filled seed (good and bad) as determined by a cutting test on the remaining ungerminated seed at the completion of the germination test. In seed testing when germination is expressed as a percent of the entire number of seed (usually 100 seed per sample, counted out from a pile as they come) the quality of the sample is expressed, to be sure, but it would be equally logical to include bits of inert matter, were they as indistinguishable from sound seed as are many "blind" seed. In drawing samples for germination tests of agricultural seed the Association of Official Seed Analysts of North America provides that broken seed larger than one-half a seed be counted as clean seed. Such a method is not suitable for obtaining material for use in studying the behavior of a given species under different environmental conditions. Here the material should be of the highest quality and as homogeneous as possible. Separation of empty seed by flotation in alcohol is suggested as being an easy method of obtaining 100 percent of the seeds with kernels. The effect of the method on the vitality of seed is also of interest in that it has been common practice in many foreign countries to color imported seed with eosin in the customs. Since a water solution was found unsatisfactory because the seed formed bunches and stuck together, alcohol (75 percent) was prescribed as a solvent by the law of 1910 in Sweden. Schotte (29) found that this treatment reduced and retarded germination 24 percent in the case of white spruce, but concluded that this loss was of little consequence in comparison to the danger of importing seed of origin unsuited to the planting site.

The purpose of the experiments reported in this paper was thus two-fold: (1) to determine with what degree of success empty spruce seed could be separated from filled seed by flotation in alcohol, and (2) the effect, if any, of this treatment on the vitality and keeping qualities of the seed.

Historical

The use of various liquids for separating good seed from impurities has been reported for many years, and botanical literature abounds with references to this method. Absolute alcohol especially has been a favorite liquid. Kienitz (13) used it to separate empty spruce seed from good, but did not report the effect of the alcohol on the good seed. Becquerel (3) showed that the thoroughly dry seed coats of certain plants were impervious to absolute

alcohol. Recently Schmidt (24) has used absolute alcohol to separate out the empty seed of *Picea excelsa*. For species of *Pinus* and *Larix* he found it less satisfactory.

The effect of alcohol on the vitality of seed has been the subject of many investigations. Nobbe (18) describes holding clover seed in absolute alcohol for four months without its having the slightest effect on the seed, but he mentions that different species vary greatly in their resistance to alcohol. He states further that seeds containing oil are damaged by "even dilute alcohol." Giglioli (9) reported the extraordinary power of *Medicago sativa* to retain its vitality when stored in absolute ethyl alcohol 834 days. In a later communication (10) lucerne seeds stored in absolute ethyl alcohol over 16 years were reported to have germinated 66.6 percent after air-drying for 12 hours.

The stimulatory effect of alcohol on seed germination has been reported by Hesse (11), who found it increased the germination of *Verbascum thapsiforme* in the dark, thus partially replacing the need for light. Seeds soaked one day in absolute alcohol and then dried, germinated 54 percent in 10 days, as compared to 17.5 percent of the controls.

Evidence of the effect of alcohol on seed is conflicting, and varies from beneficial to injurious depending on the individual species. A number of other investigations might be cited (4, 5, 6, 7, 8, 14, 15, 16, 17, 19, 20, 21, 22, 23, 27, 30, 31, 32, 33, 34, 35) reporting various effects of alcohol depending on the species and experimental conditions. Specific differences in the permeability of the seed coats seem to determine the relative resistance of different species. Most workers have found, however, that the presence of water in alcohol greatly increases its harmful action.

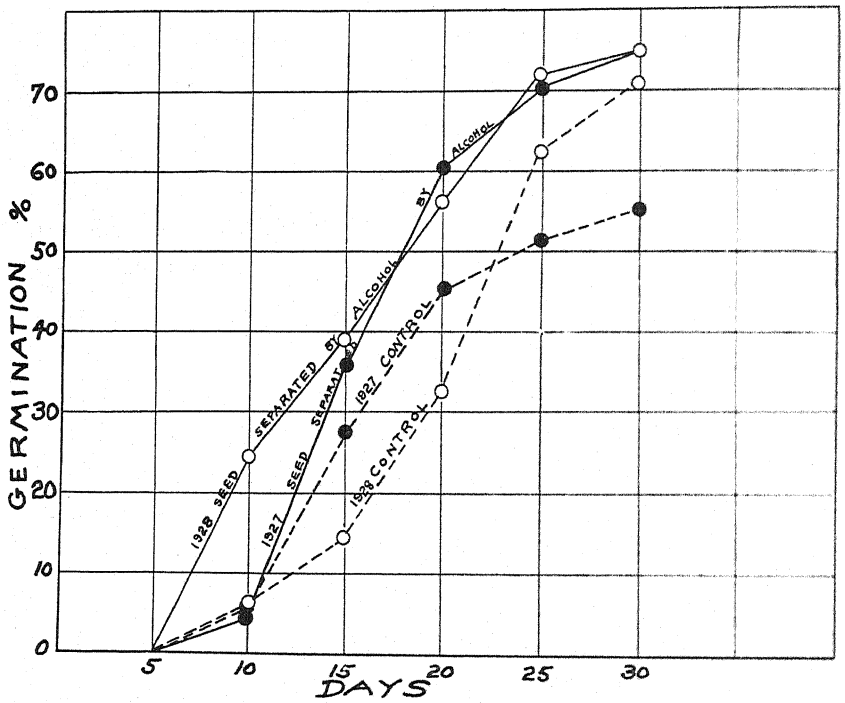
Material and Methods

The material used in the experiments reported below consisted of two lots of red spruce (*Picea rubra* Link.) seed, one from the 1927 and one from the 1928 crop. Both were collected in northern New Hampshire and Maine at an altitude of 400–500 m., in a region having a mean summer temperature of 18° C., and were extracted at the seed-extracting plant of the Brown Co. at Berlin, N. H. Until June 1929 they were kept in sealed cans at about 10° C. and thereafter in bottles at room temperature, which varied from 15° to 25° C.

Small samples of the seed were poured into a small glass dish containing absolute ethyl alcohol to a depth of 3–4 cm. The floating seed were then skimmed off with a spatula, and placed on blotters to dry. The seed which had sunk were recovered by pouring off the alcohol through a sieve, and the seed similarly dried. The operation was usually completed in two minutes, and the alcohol had completely evaporated from the seed in five minutes. Immediately thereafter samples were counted out and set to germinate; direct cutting tests were also made. A portion of the sample treated was

reserved for later tests in order to determine the keeping qualities of the treated seed. These samples were preserved in corked vials at room temperature, in the same manner as the controls. In a few cases these samples were retreated with alcohol after several months storage in order to determine the effect of repeated treatment.

For germination tests the Jacobsen type germinator was used (1). In these experiments a distance of 10 cm. was maintained between the water level and the bottom of the cotton pad, so that a uniform rate of moisture supply was insured in all tests. The temperature was different in different series, but fairly constant for any one series of comparative tests. The first experiments were performed at the Waldsamenprüfungsanstalt, Eberswalde, Germany, at 25° C., and were continued in a basement room of the Osborn Botanical Laboratory, Yale University, New Haven, Conn., where the temperature remained at about 20° C. Both temperatures fall



TEXT FIG. 1. Germination of red spruce seed immediately after immersion in absolute alcohol.

within the optimum range for the germination of red spruce.[1] Germination tests were run 30 days, and counts made usually at 5-day intervals. Upon completion of the tests all seed remaining were carefully cut open and their condition noted. They were classified according to whether they were

[1] Unpublished data.

"good," "bad," or "empty." "Good" seed were characterized by a firm white contents, sometimes with a trace of chlorophyll formation; "bad" seed were watery, or with brown, discolored or decomposed embryo and endosperm; "empty" seed were those without contents, or with dry, woody "sawdust" only. Results were then recalculated on a basis of real germination. Duplicate or quadruplicate samples of 100 seed each were used in all tests.

A limited number of catalase determinations were made on untreated and alcohol-treated seed, using the apparatus and technique of Schmieder (28) as modified by Schmidt (25). Sixty seed were used in each test, it having been found by trial that 20 red spruce seed gave too small a quantity of oxygen to be measured conveniently. Merck's "Perhydrol" hydrogen peroxid was used, acid-free, of the brand recommended for the tropics. This is more stable at high temperatures. Tests were made at a temperature of 20° C. and pH 7.0.

Results

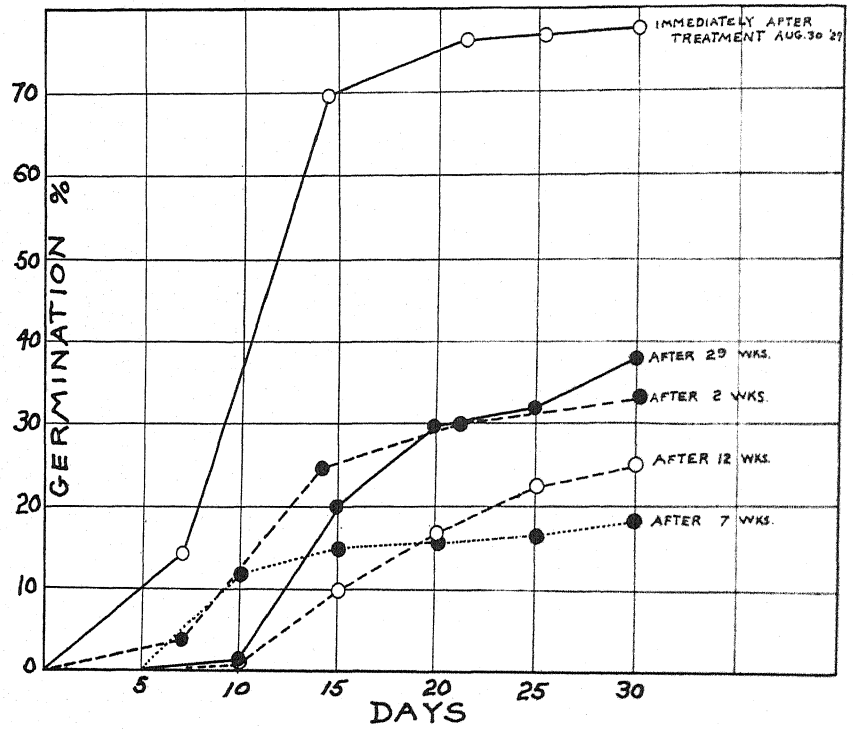
Absolute ethyl alcohol was found to float all blind seed and allow filled seed to sink. This was verified by making cutting tests on five samples from the floating seed and five samples from the sunken seed, each sample containing 100 seed. In every case all the sunken seed were found to be "full," while the floating seed contained, besides empty seed, from 10 to 15 percent "full" seed, which floated due to attachment to wing fragments, resin, etc. which lowered their specific gravity. For the purpose of experiments, this is not important, so long as no empty seed are present in the samples to be used.

The weight of 1000 seed from the "floaters" was next compared with that of an equal number of "sinkers." The weight of empty seed alone was computed to be 37 percent that of full seed. This difference is sufficient for successful air separation by means of a vertical air current (12), and it should be possible to secure red spruce seed of high quality by use of a suitable apparatus.

The results of the germination tests of seed treated with alcohol are summarized in tables 1–3, and some of the typical results plotted in text figures 1–2. Determinations of catalase activity are given in table 4 and plotted in text figure 3. Lack of space prevents the inclusion of the detailed tables. During germination nearly all seed molded to a greater or less extent, the immersion in alcohol seeming to have had no effect in killing mold spores.

Discussion

Separation in absolute alcohol was successful in eliminating nearly all the empty seed. The few remaining were almost always "solid" seed, with contents resembling "sawdust." It would thus appear that the first object of the experiments, to eliminate empty seed, could be accomplished by a



TEXT FIG. 2. Germination of red spruce seed treated with absolute alcohol, after dry storage for varying lengths of time. 1927 seed crop.

TABLE 1. *Germination of Red Spruce in percent Immediately after Treatment with Absolute Alcohol*

	Course of Germination, Days						Real Germination after 30 Days, Percent
	5	10	15	20	25	30	
1927 Seed							
August, 1929							
Treated	6	14	69.5	76	77	77	80.2
Control	0	1	14.5	14.5	14.5	14.5	65
March, 1930							
Treated	0	4.3	36.3	60.6	70.3	75	77
Control	0	6.2	27.7	45.7	52	55.2	78
1928 Seed							
November, 1929							
Treated	0	24	38.5	56.5	72	75	77.5
Control	0	6	14.5	32.5	62.5	71.5	75
March, 1930							
Treated	0	10.7	44.2	60.2	71.7	75.5	76
Control	0	0	24	45.5	59.6	67	76

NOTE: Each figure represents the average of 200–400 seeds.

TABLE 2. *Germination of Red Spruce Seed in percent, Stored Dry after Alcohol Treatment*

	Course of Germination, Days						Real Germination after 30 Days, Percent
	5	10	15	20	25	30	
1927 Seed							
2 months							
Treated	0	12	15	16	17	18	18.3
Control	0	36	52	61	64	65	67.3
3 months							
Treated	0	1.5	10	17	22.5	25	25.2
Control	0	31.5	50.5	63.5	73.5	76	76.7
7 months							
Treated	0	1.7	20	29.5	34	37.7	39
Control	0	6.2	27.7	45.7	52	55.2	78
14 months							
Treated	0	6.2	9	9.6	10.5	10.5	10.7
Control	0	57.2	64.7	66.7	67.7	69.0	69.2
1928 Seed							
4 months							
Treated	0	9.5	50	64.7	74	78.2	80
Control	0	0	24	45.5	59.5	67	76
7 months							
Treated	0	56.3	64.6	67.3	70	72.6	73.4
Control	.5	64.5	72.2	73.7	75.5	76.7	78.2
11 months							
Treated	0	59.2	71.5	74.2	76.2	78.7	80
Control	.5	64.5	72.2	73.7	75.5	77.7	78.2

NOTE: Each figure represents the average of 200–400 seeds.

TABLE 3. *Germination of Red Spruce Seed in percent, Treated with Absolute Alcohol and Stored Dry, Compared with Similar Seed Retreated*

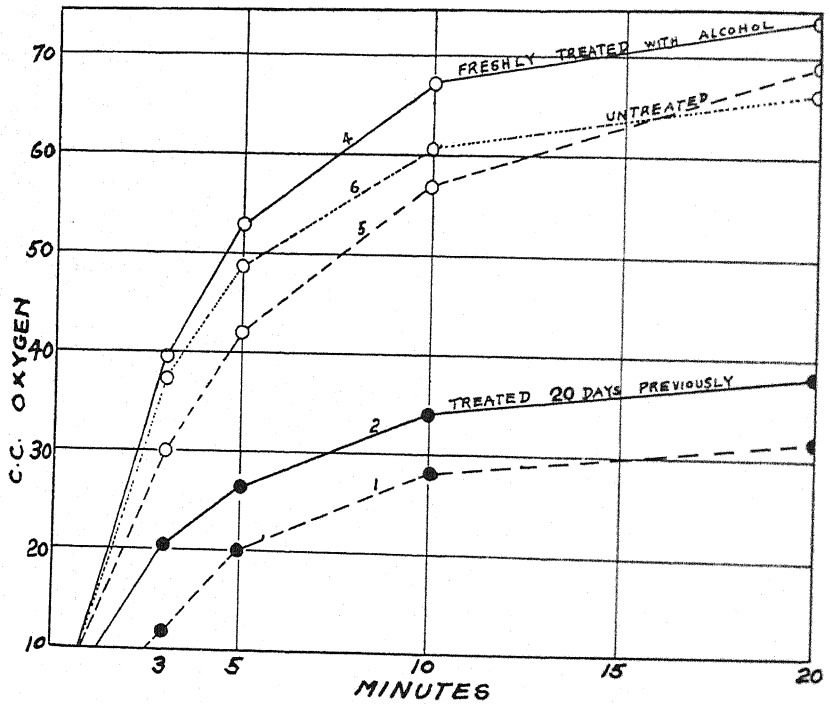
	Course of Germination, Days						Real Germination after 30 Days, Percent
	5	10	15	20	25	30	
1927 Seed							
Retreated	0	2	23.2	34	41.7	45.5	47
Treated 7 months before; no retreatment	0	1.7	20	29.5	34	37.7	39
Retreated	0	.7	1.2	1.6	3.3	3.3	3.5
Treated 14 months before; no retreatment	0	6.2	9.0	9.6	10.5	10.5	10.7
1928 Seed							
Retreated	0	45	72	77.5	77.7	80.2	82.3
Treated 4 months before; no retreatment	0	9.5	50	64.7	74	78.2	80
Retreated	0	27	34.3	37.3	43.6	44.6	45
Treated 11 months before; no retreatment	0	59.2	71.5	74.2	76.2	78.7	80

NOTE: Each figure represents the average of 200–400 seeds.

TABLE 4. *Catalase Activity of Red Spruce Seed Treated with Absolute Alcohol Compared with Untreated Seed, 1927 Seed Crop. Averages of Two Determinations of 60 Seed Each*

Treatment	Average Volume of Oxygen, in cc., Evolved after:			
	3 min.	5 min.	10 min.	20 min.
(1)* Treated with alcohol 20 days before; dry seed	12	20	28	31.5
(2) Above seed after 3 days in germinator	20.5	26.5	34	38.2
(3) Treated with alcohol 3 days before; dry seed	results unreliable			
(4) Above seed after 3 days in germinator	39.5	53	67	73.7
(5) Control, dry seed	30	42	57	69
(6) Control, after 3 days in germinator	37.5	48.5	60.5	66.7

* Numbers refer to curves in text figure 3.



TEXT FIG. 3. Catalase activity of treated and untreated red spruce seed expressed in cc. of oxygen evolved.

fairly simple and cheap procedure; in fact, a process reported to use flotation in special liquids to eliminate dirt and weed seeds has been developed on a commercial scale in the Middle West for cleaning grain. It is quite possible that further investigation may show that this method can be applied profitably in cleaning forest tree seed. So far, the writer's attempts to clean *Abies balsamea* with alcohol have been unsuccessful. No other species

have been tried. It must be recognized that but one liquid, ethyl alcohol, was used in these experiments; there may be many others which will prove superior, both in efficiency of separation and in freedom from damage to the seed.

The germination results are not wholly consistent. The data show an apparent initial stimulation immediately after treatment, but in most cases the final differences are not significant; the older lot of seed seems to have been more stimulated by the treatment than the fresher seed. Treatment resulted in a decline in vitality in storage as compared with the controls. However, in half the cases seed which showed poor germination several weeks after alcohol treatment could be revived slightly by another treatment. This was not sufficient to restore it to the level of the untreated control seed, however. Practically, the value of alcohol treatment to remove empty seed hinges on whether such a process injures the seed. The present experiments show that it does. It is also safe to assume that treatment of red spruce seed with alcoholic solutions of dyes in the customs would result in injury to the seed if it were not sown immediately.

The few catalase determinations show a consistent parallelism with the results of the germination tests. In comparing treated seed with controls, it should be remembered that the treated seed contained 100 percent full seed, while the controls contained an unknown percent (approximately 5 percent) empty seed. Unfortunately it was not possible to make more tests and to follow the course of catalase activity in the stored seed for longer periods. Taken in conjunction with the more extensive germination tests the results indicate that the effect of the alcohol was first to excite the ferments in the seed to activity; if the seed could not at once find expression for this awakened activity in germination, a weakening ensued.

Without microchemical tests nothing could be learned of the exact effect of the alcohol. No special precautions were taken to remove the last traces of water from the alcohol. It is possible that less damage would have been caused had this been done. When absolute alcohol is used there is no swelling, and presumably no immediate penetration of the alcohol. When the seed were placed to germinate soon after treatment some traces of alcohol may have been absorbed with the water, but yet have been so weak that a stimulating effect was produced rather than a harmful one. It is well known that alcohol, like many other poisons, is stimulating in weak doses and harmful in higher concentrations. The smaller and more delicate the organism the smaller the lethal dose. In the case of seed stored dry after treatment, it is probable that weaker seed, with slightly more permeable seed coats, absorbed enough of the alcohol, possibly in gaseous form, to damage the protoplasm. Where conclusions must be drawn from the average performance of a large number of individuals, it is obvious that the significant phenomenon is the behavior of the weaker and more sensitive individuals, which are the first to succumb and affect the results of the whole.

Despite the conflicting nature of the data, the combined results of germination tests and catalase determinations point to an injury from alcohol treatment which, however, is not immediate, but rather manifested by a more rapid drop in viability under ordinary conditions of storage, than in the case of untreated seed.

These variations in germinative energy and germinative capacity following treatment with alcohol, may be superimposed upon unknown periodical seasonal variations, which have been shown by Schmidt (26) to be very marked in the case of *Pinus sylvestris*, with a maximum in March to April and a minimum in the late autumn and January. This may assist in explaining the higher results obtained in March over November, with the same sample.

Summary

1. By immersing in absolute alcohol for a minute or two it was possible to remove empty seeds of *Picea rubra* Link. by flotation, and obtain practically 100 percent full seed from those which sank.

2. Seed which had been immersed in absolute alcohol, when dried and placed at once under conditions suitable for germination, germinated more promptly and completely than untreated seed.

3. Seed thus treated declined notably in viability in dry storage. In some cases germination of such seed could be improved by retreatment with absolute alcohol.

4. Catalase activity increased immediately after treatment, but dropped to one-half that of untreated seed after 20 days.

5. It is concluded that the use of alcohol for separating empty seed, and the use by customs officials of alcoholic solutions of dyes for identifying imported seed, is open to objection in the case of red spruce.

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SPECIFICITY TO PENETRATION OF THE EPIDERMIS OF A PLANT BY THE HYPHAE OF A PATHOGENIC FUNGUS

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INTRODUCTION

Specificity to attack by disease-inducing organisms has long been accepted as fact, and because of this acceptance little experimental work has been done on the problem. On the other hand, a vast amount of work has been done on the questions of immunity, resistance, and susceptibility. It is from these latter studies that information might be taken as a basis for a study of specificity to attack by disease-inducing agencies. No attempt will be made here to review this literature, for reviews are to be found elsewhere (1, 5, 7, 8, 10, 11, and 13).

It may be well to define what is here understood by the term "specificity." "Immune," "resistant," and "susceptible" are descriptive terms applied to plants within a single species in their reactions towards parasites. Massee (6) stated the situation as follows: "An *immune* plant signifies an individual of the same species as the one on which a given species of fungus is parasitic; but which . . . remains unattacked." Vavilov (8) said that "practically, the phenomena of immunity are usually connected with immunity of races and varieties, rarely with that of species." Any immunity connected with species and genera can be referred to as "specificity" against a parasite.

Usually cross-inoculations are attempted with the idea of determining the host range of a particular fungus. However, Miss Gibson (4) and Young (12) made a large number of cross-inoculations, apparently with the idea of noting only the reactions of each fungus with its experimental host. These papers are of interest in connection with the topic under discussion. Miss Gibson used the uredospores and aecidiospores of a number of rusts, and inoculated several plants as experimental hosts. The rust spores from 15 genera of plants were found to germinate on at least one of the experimental hosts used. In 18 of the 19 cross-inoculations she made, the germ tubes enter the stomata of the hosts, but in no case were the hyphae and haustoria within the experimental hosts normal. She found that normal parasitism by the fungus could not be secured on varieties of chrysanthemums resistant to the chrysanthemum rust except on the leaves of the flower stalks. She could not induce the formation of pustules by changing the mineral nutrition of young resistant plants. Neither could immunity be conferred on susceptible varieties by grafting them on resistant varieties.

Young used a large number of fungi on a long series of experimental hosts. The results to which he devotes the most attention concern plants grown under aseptic conditions in test tubes. He reports several sorts of reactions of the hosts to the various fungi used. Callosities, penetrated vertical cell walls, swollen vertical cell walls, browned internal cells, and stained walls of epidermal cells were noted. Attempts to secure infections on plants grown under ordinary greenhouse conditions were generally unsuccessful, although the plants were held in a moist chamber for a period after the inoculations had been made.

Direct experimental knowledge concerning specificity to penetration by fungous hyphae is meager, and in large part observations on resistance-susceptibility relations do not seem to be especially applicable in a study of specificity. The fact is that specificity to disease has long been accepted as fact and little or no concerted effort has been used in unraveling the complexities of this subject. In this paper it is intended to deal with the first question to arise in such a problem—namely, do fungi penetrate plants other than their specific host plants?

Microscopical Study of Penetration

The attempt was made to simulate ordinary cultural conditions as much as possible. Therefore, most of the plants were grown in ordinary greenhouse soil in four-inch pots. In our first experiment, buckwheat, bean, and cotton were grown in nutrient solutions. The leaves of the plants were inoculated with suspensions of spores of *Colletotrichum circinans* after the plants were growing well and had set a number of true leaves. Stock cultures of the fungus were carried on bean plugs.

C. circinans is considered to be limited in its host range to the onion (*Allium Cepa*), the shallot (*A. ascalonicum*), and the leek (*A. Porrum*). It can also become established on fruits of apple (9). Because of its limited host range and because of ease in culturing the organism, *C. circinans* was used exclusively as the experimental pathogen in these studies.

The suspensions of spores were prepared by pouring 10 cc. of water or 10 cc. of 1 : 10 prune juice in a culture of the fungus and mixing thoroughly. Drops of this mixture were placed on either or both sides of leaves which appeared to be in the full vigor of growth. The place of the inoculation was outlined with India ink. Immediately after inoculation the plants were placed in a large moist chamber and held there for three days.

The upper part of the moist chamber was made of glass. A high humidity was maintained by spraying water into the chamber. Although the chamber was by no means air tight, during the hottest parts of warm days the temperature tended to rise above 30° C. These excessive temperatures were counteracted by hanging curtains of cheesecloth in the chamber and by having a layer of cold water flowing over the top of the chamber.

After the inoculated plants had been held in the moist chamber for

three days, they were removed and allowed to dry for about an hour. The drying completed, the squares of inoculated tissue were cut out and fixed in formal-acetic-alcohol solution for 24 hours. Dehydration, infiltration, and embedding processes were conducted as is usual for such work.

Microtome sections were cut from 6 to 11 microns thick. The best results were obtained with sections 8 microns thick, and most of the material was so sectioned. Staining was by a slight modification of Dickson's method (2). Other staining methods were tried but success with them was more or less indifferent.

In brief, the technique used (*a*) did not permit the sterilization of soil, which would eliminate the influence of the flora of the soil, (*b*) did not permit sterilization of surfaces of the plant which would remove the possibly very important factor of competing organisms (3), (*c*) did not permit growth of plants under excessively abnormal conditions, and (*d*) did not permit the use of excised portions of the plant, the inoculations being made on actively growing leaves. Under natural conditions, specificity against attack by fungi seems to be general, so that it would seem necessary in a study of specificity to maintain any limiting factors which might possibly prevent parasitism by any organisms. It is obvious that any technique which might eliminate limiting factors (however good that technique may be usually in phytopathology or in bacteriology) can be of little value in a study of specificity. It would seem that under natural conditions any relation of an organism to a host, including its "natural" host plants, would have to take place under competitive conditions, and not under the conditions of pure culture and optimum environment found in some of the best phytopathological technique.

The principal object in view was to find whether or not penetration pegs of *C. circinans* enter leaves of experimental plants. The first method of attack in this study was the microscopical examination of sectioned and stained materials.

The most noticeable reaction of most of the experimental hosts used was the formation of heavy red-staining (with magdala red) thickenings of the outer walls of epidermal cells in some of the cases were appressoria were in contact with the walls. Plate I, figures 3, 4, 5, 6, 8, 9, and 10, show some of the typical forms and sizes of the thickenings found. As is seen from the illustrations, there is no definite shape for these thickenings, but they occur only in association with appressoria. There is a strong tendency for appressoria to form at or near the junctures of two or more epidermal cells. The plants showing red wall thickenings were buckwheat (*Fagopyrum esculentum*), bean (*Phaseolus vulgaris*) var. Kentucky Wonder, cotton (*Gossypium hirsutum*), field pea (*Pisum sativum* var. *arvense*), soy bean (*Soja max*), tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana tabacum*), cucumber (*Cucumis sativus*), squash (*Cucurbita maxima*), pea (*Pisum sativum*) var. Dwarf Telephone, beet (*Beta vulgaris*), mustard (*Brassica alba*), cabbage

(*Brassica oleracea* var. *capitata*), California poppy (*Eschscholzia californica*), yellow lupine (*Lupinus luteus*), and white clover (*Trifolium repens*). Onion (*Allium Cepa*) did not exhibit these wall thickenings.

Lima bean (*Phaseolus lunatus macrocarpus*) presented the most distinctive type of wall thickening of any of the plants examined. In the lima bean all the wall thickenings observed were bulb-like in shape and usually did not extend much farther than the limits of the appressorium. They were all of a hyaline translucent nature, none of the usual red-staining wall thickenings being noted. In connection with many of these peculiar formations the appressoria appeared to be ready to put out infection pegs, but usually an actual penetration was not to be observed. In figure 10 there is what appears to be the usual type of wall thicking as well as the beginning of such a thickening for the lima bean. The normal outer walls of the lima bean are little or no thicker than the walls of the mesophyll cells.

In addition, buckwheat, soy bean, tobacco, tomato, and pea in particular had many collapsed epidermal cells. We do not know whether the appressoria on these cells caused their collapse or not. These cells stained red also, and in many cases penetration pegs were disclosed growing through the walls (fig. 2).

In all the plants mentioned above, except lupine and onion, the vertical walls of the epidermal cells also reacted in some cases to the presence of appressoria (figs. 3, 4, 5, 6, 7, and 9). Generally the vertical wall thickenings also stained red. Figure 7 shows a case in which the vertical wall is darker and thicker than the normal walls. Such cases are often interpreted as intercellular penetrations.

Incipient or even well-defined penetrations were seen in all the above plants, figures 6, 10, and 11 being typical of such phenomena. Pea (fig. 1), California poppy, nasturtium (*Tropaeolum majus*), and green leaves of onion all showed apparently normal hyphae ramifying throughout the tissues of the leaves. In some cases the tissues were more or less disintegrated although the plants had been inoculated for only three days.

Reference to the illustrations will show that not all appressoria on the same plant will cause the same reactions. For example, cases were seen in which one of two appressoria on a single epidermal cell would send a penetration peg into the cell, while the other appressorium was associated with no apparent host reaction. Figures 2 and 6 show similar cases of two different reactions on the same cell.

Only small amounts of lettuce (*Lactuca sativa*) and castor bean (*Ricinus communis*) were available for microscopical study. On the first named plant, only a very few appressoria were seen, while none were seen on the castor bean. More material should be examined before a conclusion is reached as to whether or not there is little or no formation of appressoria and no penetration in these plants by *C. circinans*, or whether it just happened that examination was made of material from which the inoculum had accidentally been removed.

In order to check on the above described fixed material and to see whether or not the possible presence of artifacts was entering into the observations, the following experiment was performed, using *Bryophyllum crenatum* as the experimental host. The methods of inoculation and incubation were as usual. Inoculation drops were of water and of 1:10 prune juice. Free hand sections of the inoculated leaves were prepared and examined in temporary water mounts. The same experiment was repeated except that the plants remained in the moist chamber five days. Penetrations were seen in all the different inoculations regardless of the kind of inoculation drop used or the length of time the plants were left in the moist chamber.

The fact that magdala red, in the staining process used, stains the wall thickenings associated with appressoria of *C. circinans* red in all cases, except lima bean, indicated that materials of similar nature occur in the wall thickenings, regardless of the species of plant in which they occur. In order to test this assumption of the constancy of the materials in wall thickings, use was made of simple microchemical tests.

The results of the microchemical tests are given in table 1. Again, with

TABLE 1. *Results of Microchemical Tests to Determine Whether or not the Materials in Wall Thickenings Are Similar in All Plants*

Plant	Tests with Sudan IV for Cutin	Tests for Cellulose	Tests for Lignin
Buckwheat	+	−	−
Cotton	+	o	−
Tomato	+	−	−
Tobacco	+	−	−
Cucumber	+	−	−
Lima bean	−	−	−
Squash	+	−	o
Beet	+	o	−
Cabbage	+	o	−
Clover	+	o	−

+ = positive result; − = negative; o = no observations.

these tests lima bean was the only plant found to give a specific color reaction at the wall thickenings. Although the intensity and shade of red caused by treatment with Sudan IV is not exactly the same in any two plants, the results are definite enough to show the probability of similar substances in all the wall thickenings except those of lima bean. Two different tests were used to determine the possible presence of lignin. Negative results were obtained in all cases. Negative results were also obtained in cases in which tests for cellulose were made.

These studies do not definitely demonstrate that the wall thickenings are of cutin or of cutin-like substances. Indications, however, are that in all cases, lima bean excepted, the nature of the wall thickenings in the various plants is similar. No exact conclusion as to the substance in the

wall thickenings is made. That the constitution of all wall thickenings observed is similar (lima bean excepted) is based, then, on (*a*) similar staining reactions with magdala red, (*b*) similar color reactions upon treatment with Sudan IV, (*c*) the negative evidence that no positive reaction for lignin was observed, and (*d*) the negative evidence that no positive reaction for cellulose was detected.

Statistical Study of the Reactions of the Epidermal Walls to the Presence of Appressoria

Many workers when reporting the results of their studies concerning the numbers of penetrations or other host-parasite relations use only very general terms. Such terms as "freely" and "generally" are often difficult of interpretation. It seems necessary that comparative numbers or percentages be given if a true picture of the extent of a reaction is to be secured. Some writers have given such data. It was in an effort to find with some degree of comparative exactness what had happened in our materials that a representative survey of some of the host-parasite relations was attempted on our materials.

The method was to examine every fourth section on a slide. Enough slides were examined to give what was believed to be sufficient numbers of appressoria to be statistically significant. All examinations were made at a magnification of 660 diameters. With such a magnification a fairly accurate and rapid survey can be made, but there is always the chance of mistaking a slight contortion of the host cell for an infection peg when using such low magnifications.

The manner of classification is as follows: All the appressoria resting on the leaf, or obviously connected with the leaf, were counted and recorded as "number of appressoria." Then all the cases where appressoria were distinctly in connection with red-stained thickenings of the outer wall of the epidermis were counted and computed as "percent of appressoria connected with wall thickenings" or in table 2 "percent of wall thickenings." Illustrations of this condition are to be found in figures 3, 4, 5, 9, 10, and 11. Although the thickenings in the lima bean did not stain red, they were classified as "wall thickenings," no distinction being made between the red-staining thickenings and the hyaline ones.

The reaction listed as "percent of vertical wall thickenings" in table 2 is figured in figures 4, 5, 6, 7, and 9. In such cases the joining walls between two epidermal cells become noticeably thicker than the normally green-staining walls. These abnormal walls are usually red. However, figure 7 illustrates quite well a condition where the affected vertical cell wall is merely thicker and darker than the normal walls. Due to the fact that penetration hyphae are so small and indefinite in outline, it is extremely difficult to determine accurately whether such cases as those in figure 7 are actual penetrations or purely a reaction of the cell walls to the presence of the appressoria.

TABLE 2. *A Summary Table of the Comparative Percentages of Occurrences of Wall Reactions of Epidermal Cells of Experimental Hosts to the Presence of the Appressoria of C. circinans, to the Total Number of Appressoria Observed. See Text for the Bases of Classification of the Different Kinds of Reactions*

Plant *	No. of Appressoria	% of Wall Thickenings	% of Vertical Wall Thickenings	% of Penetrations	% of Appressoria on Collapsed Cells	% of Stomatal Penetrations	% of Non-reactions
Buckwheat	2202	49.1	6.9	0.9	12.3	—	35.7
Bean (Kentucky Wonder)	821	0.7	9.1	2.4	0.0	0.1	87.7
Cotton	304	32.8	8.8	0.6	0.3	0.0	59.9
Field pea	393	9.4	8.1	1.7	0.2	0.0	84.7
Soy bean	1229	26.5	12.2	3.3	11.2	0.0	53.7
Tomato	706	39.5	2.8	1.9	18.4	0.1	40.0
Tobacco	434	38.2	3.4	0.6	6.2	0.0	51.3
Cucumber	960	4.1	6.6	1.7	0.0	0.0	89.4
Lima bean (old)	180	27.7	0.0	2.2	0.0	0.0	70.0
Lima bean (young)	219	6.3	0.4	1.3	0.0	0.0	91.7
Pea (Dwarf Telephone)	919	4.3	0.2	0.5	14.0	0.0	80.8
Beet	825	39.0	6.3	1.0	0.0	0.0	56.3
Mustard	669	1.8	7.0	3.1	1.0	0.0	87.4
Cabbage	979	17.9	2.5	1.8	0.0	0.0	78.4
Onion	324	0.0	0.0	11.1	0.0	0.0	88.8
Lupine	641	0.4	0.0	0.8	0.0	0.0	98.7
Clover	179	18.5	6.1	7.2	0.0	0.0	68.7
Squash	714	4.0	1.1	0.9	0.2	0.0	93.5

* Cells of nasturtium were so collapsed that it was difficult to determine the real relations between appressoria and host. The sections of the poppy material were so irregular that it was difficult to count appressoria without repetitions.

The situations shown in figures 3, 4, 6, 8, and 9 will serve well to mark the distinction between "wall thickenings" and "vertical wall thickenings." In figures 4, 6, and 9 the classification in each case would be a "wall thickening" and a "vertical wall thickening." In other words, these three appressoria would be counted twice. It is because of the few cases of this nature that the percentages in the several classifications often total slightly more than 100 percent. The illustrations in figures 3 and 8 show borderline cases. This sort of a reaction was generally classed as a "wall thickening."

From the discussion immediately above it is readily seen that the classifications used are entirely arbitrary, and that much is left to the judgment of the observer in his distribution of the reactions into their several classes. Despite the objections which might be raised, it is believed that a degree of accuracy in classification was attained so that the results can not be seriously questioned and that they give an adequately clear picture of the conditions found in our materials.

In some cases the epidermal cells were collapsed. We are not prepared to say whether the presence of the fungus caused the observed collapse or whether the collapse was due to some other cause or causes. Therefore, we

have been content to say merely that the appressoria in such cases are "on collapsed cells." A typical situation of this nature is shown in figure 2 taken from a leaf of soy bean. Because of the uncertainty as to the cause of the collapse of these cells no attempt will be made to discuss their relation to specificity.

Large numbers of appressoria were not associated with any of the above described reactions. Such appressoria are listed in the column headed "no reaction observed."

Reference to table 2 will immediately show that there is no definite relation between the single species of fungus used and the number and kinds of reactions associated with the appressoria of the fungus. The numbers of reactions seem to be dependent to some extent on the species and condition of the plant being attacked. This is not to say, though, that there is absolute specificity to penetration by the hyphae of fungi.

First, considering the most obvious condition, this work tends to confirm the general opinion that directly penetrating fungi seldom enter stomata. The absolute number of stomata found to be penetrated in our material seems to be greater than that reported by other workers. In the thousands of host-parasite relations that Young (12) must have examined, he found only two cases of stomatal penetration. Not only is the absolute number observed by us greater, but the percentage of cases would also be greater.

Our study did not reveal large numbers of penetrations. Considering these data one could not well say that there is specificity to penetration on experimental hosts. Excluding the percentage of penetration in onion leaves, we find that with one exception, clover, all the percentages lie between 0.5 and 3.3. Walker (9) had already observed that young onion plants are damped off by *C. circinans*. Results from other workers are not at hand giving the percentages of penetrations of onion scales by *C. circinans*. Dr. Walker, in a verbal communication, gave it as his opinion that comparatively few penetrations occur in the onion bulb scales in comparison with the number of spores of *C. circinans* present. It has already been pointed out that there is a very high percentage of germination of spores of *C. circinans* on a variety of leaves. In order to find the percentage of penetration in onion bulbs the following experiment was undertaken. An entire white onion bulb was used, the outer dry scale being removed. The onion was placed in a glass moist chamber and inoculated by pouring over it a heavy suspension of spores of *C. circinans* in water. Conditions of inoculation and treatment prior to examination were similar to those for *Bryophyllum* (above). Examination was by Walker's method. Appressoria were so numerous in places that it was difficult to find individuals upon which accurate observation might be made. A systematic survey of the material was made, and the first 200 appressoria placed by themselves were counted for the number of cases of penetration with which they were associated. Twenty-nine penetrations (14.5 percent) were found. Another similar

experiment gave confirming results. Therefore, if our assumption that the percentages of penetrations in onion scales are comparatively low is correct, then the percentages given in table 2 can not be considered unduly low for experimental hosts; and this fungus can not be said to show a high degree of specificity in its host range as far as penetration is concerned.

Turning now to the percentages of vertical wall thickenings, there is found to be a rather remarkable constancy in the reactions of these plants toward the appressoria of *C. circinans*. Again disregarding onion, in only two plants, mature lima bean and lupine, are there no thickened vertical cell walls. All other plants show frequencies of this reaction equalling 0.2 percent to 12.2 percent of the appressoria observed on them, most of the percentages falling between 2.5 and 9.1. From such comparatively constant results over a wide range of species, it can hardly be said that this reaction is specific to a few plants.

The same general condition can be found in that class of reactions designated as "wall thickenings." In these cases the reaction is much more pronounced in its individual appearance than the others just discussed. There is a wider range in the percentages of occurrence of this reaction—0.4 to 49.1—than in the others. Again it can be said that there is no absolute specificity regarding this reaction (except onion) for most of the percentages lie between 4.0 and 39.5.

The specific reaction as regards the kind of wall thickening in the lima bean has already been pointed out.

So far exception has been made concerning the green leaf of onion. That penetration by *C. circinans* is not limited to a few species of *Allium* will be shown in more detail in the following sections. Of the hosts studied, onion is the only one showing no thickening of walls and vertical walls. In that way it is specific in its reaction to *C. circinans*. But in other respects (under the conditions used) it is similar to other plants in its reactions except in the absolute percentages of occurrences of those reactions.

The Viability of Penetrating Hyphae

In this part of the work, the inoculum was either dropped on the leaves or the leaves were dipped into a suspension of spores. After inoculation the treatment was as follows: Plants were held in the moist chamber three days, after which the leaves were allowed to dry for an hour. Then the leaves were surface sterilized for either $\frac{1}{2}$, 1, 3, or 4 hours in a solution of chlorinated lime. The sterilized leaves were put in sterile petri dishes and covered with prune agar. It was considered that each colony of *C. circinans* which appeared in the plates came from a hypha not killed by the sterilizing fluid because the hypha was in the leaf.

That the sterilization employed is probably efficient is shown by the very small numbers of contaminants found on the leaves in the plates. In addition, in a control experiment, the spores of *C. circinans* were germinated

on glass cover slips. After germination the cover slips and germlings were put into the sterilizing fluid and then covered with prune agar, just as the leaves had been treated. The germlings on the cover slips were killed by even the shortest period of sterilization.

The results from the platings from the leaves are given in table 3. With the exception of lettuce, all the plants gave positive results with the technique used. In the first trials with cabbage and castor bean, no colonies of the fungus were plated out. This observation conforms to a previous one that in some of the fixed material of cabbage and all of the material of castor bean no appressoria were found at all. Similar results were obtained with some plants that had been inoculated only 26 hours before sterilizing the surfaces of the leaves.

TABLE 3. *Plants Inoculated with Suspensions of Spores of C. circinans, Held in the Moist Chamber for 3 Days and Leaves then Surface Sterilized with Chlorinated Lime for Different Lengths of Time but without Harming the Internal Hyphae. Numerators Indicate the Numbers of Colonies Found in the Plates, and Denominators Indicate the Numbers of Pieces of Surface Sterilized Leaves Used for Plating Out*

Plant	Inoculation Drop							
	1 : 10 Prune Juice				Water			
	Duration of Sterilizing Process				Duration of Sterilizing Process			
	½ hr.	1 hr.	3 hr.	4 hr.	½ hr.	1 hr.	3 hr.	4 hr.
Lettuce ‡	—	0/1	0/1	—	—	0/1	0/1	0/4
Castor bean 19	—	0/2	0/4	0/14	—	0/2	0/4	0/14
Castor bean 33	—	11/19	3/36	—	—	4/24	0/39	—
Beet	—	0/2	2/4	0/7	—	0/3	0/3	0/15
C. Egyptian beet	—	*	20/36	—	—	‡	0/7	—
D. D. Red beet	—	*	*	—	—	*	*	—
Cabbage 19	—	0/1	0/1	0/2	—	0/2	0/4	0/9
Cabbage 22	19/12	9/16	3/18	—	3/14	0/16	1/31	—
Cabbage 33	—	*	†	—	—	*	2/27	—
Lupine	—	4/4	0/5	2/10	—	4/4	0/5	0/11
California poppy	—	5/5	3/4	1/6	—	2/2	0/3	0/3
Clover	—	2/2	2/4	4/8	—	4/4	0/4	2/8
Alfalfa	6/7	3/4	7/9	—	9/5	12/11	9/16	—
Mustard	8/12	1/4	2/14	—	31/13	21/11	13/15	—
Radish	22/7	4/8	0/14	—	16/7	11/12	1/9	—
Bean (Kentucky Wonder)	13/12	9/10	0/5	—	—	—	—	—
Lima bean	28/16	28/19	11/26	—	16/15	12/10	2/5	—
Squash	19/12	4/16	0/20	—	31/11	6/11	0/22	—
Onion	8/8	1/11	6/17	—	21/8	13/7	11/11	—
Sunflower	—	10/5	8/14	—	—	5/5	1/12	—
Morning glory	—	15/6	36/15	—	—	36/13	65/25	—
Pea (Dwarf Telephone)	—	8/6	1/6	—	—	—	—	—
Nasturtium	—	5/4	1/7	—	—	—	—	—
Cucumber	—	20/4	25/9	—	—	8/7	1/4	—
Tomato	—	*	†	—	—	*	†	—

* All the plates contained many ascervuli on the leaves, usually the leaves being almost black with the ascervuli.

† Some of the plates contained many ascervuli on the leaves.

‡ No observations made.

This series of plating out experiments can be considered as no more than indicative that all penetrating hyphae do not die soon after entering plants other than their ordinary host plants. These results also tend to confirm the microscopical observation that penetration occurs in most plants within three days after inoculation, and that in some of the plants turgid, apparently normal hyphae are to be found.

Viability of C. Circinans in Leaves Remaining Attached to Experimental Hosts

Microscopical observations and plating out experiments having indicated that living hyphae of *C. circinans* are to be found in leaves of experimental hosts, it was decided to try a series of preliminary experiments to see whether or not the fungus could live long enough in leaves to come to sporulation.

The procedure for these experiments was as follows: The plants were inoculated and held in the moist chamber for three days in the usual manner. Then they were held under greenhouse conditions for from five to ten days. During this period all the watering was done at the soil surface to prevent a periodic wetting of the leaves. Then the plants were returned to the moist chamber for two or three days. This treatment forced the appearance of the fruiting bodies on the leaves. However, often a few scattered ascervuli could be found without returning the plants to the moist chamber. Again the plants were returned to greenhouse conditions and the leaves finally microscopically examined at the end of another three to seven days to determine the possible presence of ascervuli of *C. circinans*.

All the plants used were young (many still retained functioning cotyledons) and apparently healthy and vigorously growing (except possibly radish). Thus any attack which might be observed would, at least in the first stages, be parasitic in nature. All results were taken from the true leaves of the plants and if the fungus was found on the cotyledons note is made of that fact in the proper place. In some cases the fungus was reisolated from the diseased leaves, and white onion bulbs in the moist chamber and white onion sets in sterile soil were inoculated with the recovered fungus. In such tests the onions were all heavily infected with smudge, demonstrating that the ascervuli observed were those of *C. circinans*. In addition, when mounts of the leaves supporting a growth of *C. circinans* were made, very seldom were spores of other fungi observed.

The results of these several experiments are to be found in table 4. It will be noticed that at times the numbers of plants with symptoms are greater than the numbers of plants actually observed to have ascervuli of the fungus on them. This is due to the fact that the notes on symptoms were taken independently of and before the observations on ascervuli. That the results on the symptoms and ascervuli conform as closely as they do indicates that peculiar and definite symptoms are caused by the fungus. Also, it should be remembered that symptoms caused by *C. circinans* on these

TABLE 4. *Data on the Parasitism of C. circinans on a Number of Common Economic Plants. See Text for Details*

Plant	Inoculation Drop								
	Control			1 : 10 Prune Juice			Water		
	No. of Plants	No. of Plants with Symptoms	No. of Plants with Ascervuli of *C. circinans*	No. of Plants	No. of Plants with Symptoms	No. of Plants with Ascervuli of *C. circinans*	No. of Plants	No. of Plants with Symptoms	No. of Plants with Ascervuli of *C. circinans*
Buckwheat	7	0	0	8	2	2*	11	2	1
Bean (Kentucky Wonder)	3	0	0	6	3	2	6	2	0
Cotton	3	0	0	5	2?	1	2	0	0
Field pea	4	1?	0	4	4	3	3	3	3
Soy bean	4	3?	0	7	3?	0	3	1?	0
Tomato	1	0	0	2	2	2	2	2	1
Tobacco	1	0	0	3	0	0	3	0	0
Cucumber	8	0	0	16	3?	3	22	5?	1
Lima bean	3	0	0	5	1	1	7	1?	0
Squash	6	0	0	11	0	1	10	0	0
Pea (Dwarf Telephone)	8	0	0	14	14	(3)†	14	14	(3)
Mustard	15	0	0	21	13	(6)	36	13	(7)
Cabbage	3	0	0	3	3	2	3	3	3
Castor bean	3	0	0	4	0	0	3	0	0
C. Egypt. beet	12	0	0	7	5	5	3	1	1
D. D. Red beet	17	0	0	47	0	0*	27	2?	1*
Alfalfa	18	0	0	21	1?	0	4	4?	4
Radish‡	12	0	0	10	7	(2)	15	9	6
Morning glory	3	1?	0	11	0	0*	7	0	0*
Sweet pea	—	—	—	7	—	5†	7	—	5
Bean (Golden Wax)	3	0	0	8	4	0	5	4	0
Pea (Alaska)	9	0	0	11	11	(3)	11	11	(3)†
Radish‡	7	0	0	12	11	(9)†	10	10	(8)

* One to a number of plants also had cotyledons infected by *C. circinans*.

† One or more plants were killed by the parasitism of *C. circinans*.

‡ These plants were not growing vigorously soon after the experiment started. *C. circinans*, then, may have grown saprophytically in these cases.

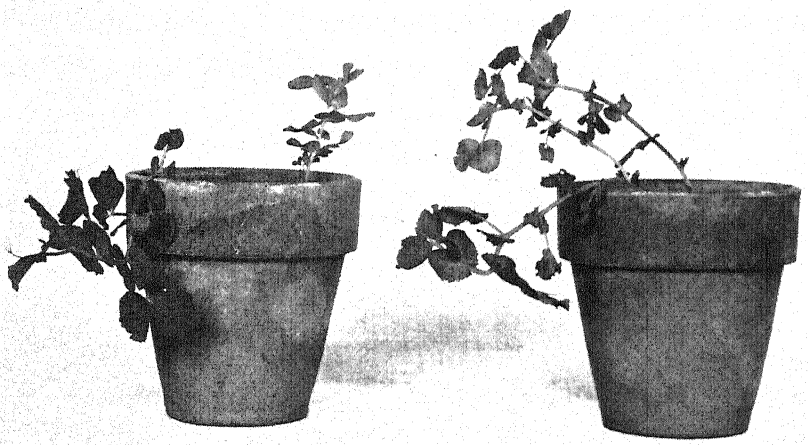
Numbers in parentheses indicate that only that many plants of the total showing symptoms were microscopically examined, and that all which were microscopically examined had ascervuli of *C. circinans* on them.

plants have never been reported before, so that possibly absolute agreement could not be expected on the first observation. The results given under symptoms are exactly as the notes were taken, cases of doubt at the time of taking the notes being indicated by a question mark.

In general, it will be seen that a number of plants are attacked by the smudge organism when one or both kinds of inoculation drops were used. In some cases the attack was so severe that the death of the host ensued. As these experiments may not be considered as more than preliminary in nature, it is entirely possible that varieties of plants that here appear not to tolerate the fungus long enough for it to sporulate on them will on further trial show the usual symptoms.

In another experiment both Dwarf Telephone peas and California poppy were subjected to rigorous conditions of moisture and temperature. The inoculation drops were of water, of 1: 10 prune juice, and of a soil extract. All plants were diseased and some of the peas were killed.

Incidental experiments on peas other than those reported in table 4 but using similar technique showed that peas were uniformly attacked by *C. circinans* under the conditions already described. To see whether or not a three-day period of moisture immediately following inoculation is necessary for parasitic attack on peas, the following experiment was conducted with young vigorously growing Dwarf Telephone peas. Plants used in some of the previous experiments were older, but nevertheless apparently healthy and vigorous. Of the 13 pots of plants available, seven were inoculated with spores suspended in 1: 10 prune juice, and six were inoculated with spores suspended in water. All were then placed in the moist chamber. At the end of 24 hours, four pots (two pots for each kind of inoculation drop used) were removed to greenhouse conditions; at the end of 48 hours four more pots were removed; and at the end of 72 hours the remaining pots were returned to greenhouse conditions. Almost immediately the typical wilting and shriveling of the leaves appeared. Text figure 1 shows plants



TEXT FIG. 1. Plants in pot at the right 4 days after being inoculated with spores of *C. circinans* suspended in 1 : 10 prune juice. The infected leaves are wilted and shriveled. Uninfected plants of the same age are in the pot at the left.

inoculated with spores suspended in 1: 10 prune juice. These plants were held in the moist chamber two days and the photograph taken two days later, i.e., four days after inoculation. After being held under greenhouse conditions 12 days, the plants were returned to the moist chamber for three days to force the appearance of the fruiting bodies. Eight days after being

returned to the greenhouse the second time, microscopical examination was made of the leaves with the results shown in table 5.

TABLE 5. *Virulence of the Parasitism of C. circinans on Dwarf Telephone Peas under Different Conditions of Moisture and with Different Media for the Suspension of the Spores for Inoculation. Plants Were Held Alternately in the Moist Chamber and in the Greenhouse. Not More than Two Regions on Each Plant Were Examined to Determine the Presence of the Ascervuli of C. circinans*

Initial Period in Moist Chamber	Inoculation Drop	No. of Plants	No. of Plants with Ascervuli of *C. circinans*	No. of Plants Killed by *C. circinans*
1 day	water	6	4	0
1 day	prune juice	5	5	0
2 days	water	9	9	1*
2 days	prune juice	7	7	2
3 days	water	7	7	0
3 days	prune juice	8	8	2

* Death of this plant may have been due to other causes than parasitism by *C. circinans*.

An attempt to induce parasitism on Dwarf Telephone and Alaska peas when the plants were held under greenhouse conditions throughout the entire experiment gave negative results.

Also, attempts to infect the same varieties of peas by *C. circinans* placed in the soil resulted in failure. Pea seeds were germinated in sterile soil. Each day suspensions of spores of *C. circinans* were added to some of the pots containing the germinating seeds. After these inoculations of the soil the pots were held under the usual greenhouse conditions (including watering). Regardless of whether the inoculation was made one, two, three, four, or five days (all plants were above ground at the end of five days) after planting the seed, typical lesions did not form on the above-ground parts of the plants.

Sterile soil in pots was inoculated with cultures of *C. circinans* worked into the soil. One-third of the pots were held outdoors during the month of February; one-third were held in a warm dark room for the same period and received no water; and one-third were placed in greenhouse conditions where they were watered every day for the same period. It was hoped that under one or more of these conditions the fungus would multiply in the soil, making a heavy inoculum. At the end of the month, Alaska and Dwarf Telephone peas were planted in equal numbers of pots from each condition, and all the pots were placed in the usual greenhouse conditions. No symptoms or ascervuli of *C. circinans* were found on the roots or above-ground parts of any of the plants. All the cotyledons were badly infected, proving that the fungus was still living in the soil. Whether the attack on the cotyledons was saprophytic or parasitic and saprophytic we do not know.

A description of the symptoms caused by *C. circinans* on the leaves of peas under the conditions employed in our studies follows. Within two to

five days after inoculation, infected leaves will suddenly wilt and curl. They dry rapidly and soon become somewhat brittle. If the killing is rapid the leaves retain part of their color, at least until the latest stages of the attack. Leaves killed more slowly change to any one of a number of shades of brown. This brown color is distinct from the yellow or buff color of leaves dying of physiological old age. Also, "naturally" dying leaves are seldom as much contorted. As the fungus becomes well established, groups of the black ascervuli can be seen by close examination, and in extreme cases the former leaf becomes a tightly rolled black mass of the fungus. Petioles, stipules, stems, and tendrils are also readily infected. At times plants are apparently killed by the attack of this fungus. It appears that most of the damage results from the primary inoculation; spread of the fungus from one part of the plant to another or through the plant has not been demonstrated. In a large number of cases, if the plant is not entirely killed, a new shoot will grow from the first or second node above the ground line. Although the upper part of the plant is just barely living, the new shoot apparently is unaffected by the fungus. Usually the leaves on the new shoot are a lighter green than those on the older stem. Text figure 1 shows the condition of the plants in an early stage of the disease.

Discussion

It has been stated by some investigators that certain tropisms are of general occurrence and are of great importance in determining the likelihood of the penetration of a plant by a fungus. If the importance of tropisms in this relation is to be accepted as a generality, it would seem, except in rare cases, that these tropisms should make possible the penetration of almost any plant by practically any fungus. But such penetrations do not seem to occur under "natural" conditions.

The attempt to explain the inhibition of hyphal growth within a plant by assuming the presence of a toxin in the host would seem to be of little help when the evidence of predisposing factors is considered. In other words, if it is true that a plant is easily attacked under wet conditions, it might justifiably be asked why the toxin is inactive at that time. The mere possibility of its dilution during the brief interval of predisposition will not explain its inaction after this short interval of predisposition. Furthermore, after normal conditions are restored, the fungus often normally continues to grow but why it should do so in the presence of the supposed toxins is not clear. On the other hand, if predisposition is of such vital importance, it would seem that more plants than have thus far been reported would be attacked by "foreign" organisms.

There are reports that nutrients, exosmosed products, and staling products are important in relation to the reaction of fungi. One is led to believe that these factors and their influences are quite commonly present. Spores germinate and appressoria form under very diverse conditions on many

host plants. Taken altogether, then, one infers that the necessary conditions for infection are present. That is to say, the lack of conditions for the germination of spores and the formation of appressoria is not a limiting factor in the amount of penetration of non-host plants by fungi. However, few penetrations of non-host plants under ordinary conditions have been reported.

Whether the mechanism of the entrance of fungi into a plant is by means of enzyms, mechanical pressure, or both, it seems that more cross-inoculations should be successful. To assume that the cuticles of hosts are different is to say that the mechanical pressure theory holds only in special cases. On the other hand, it is likewise inconsistent to say that the chemical composition of cuticles differs qualitatively according to the plant. To maintain this position, four facts must be successfully controverted. (*a*) Distinctly different enzyms have not been found for each supposedly different cuticle that has been penetrated by fungi. (*b*) Single strains of fungi have been shown to penetrate a number of different cuticles and artificial membranes. (*c*) It has not been shown that predisposing factors change either the compositions of the supposedly different cuticles or produces the enzymic actions necessary to soften each of these cuticles. (*d*) Fungi penetrate a tissue under one set of conditions but not under other circumstances.

In the numerous papers on resistance and susceptibility, attempts at correlation of morphological characters, chemical differences, and metabolic changes with resistance and susceptibility have been made. In many such cases no significant correlations have been found. In those instances where differences have been found, there is usually a wide range of conditions on either side of the mode which still permits the plant to be resistant or susceptible as the case may be. It is difficult to believe, for example, that other plants than the few reported do not have approximately the same amounts of pentosans that corn seedlings susceptible to blight have. Apparently, though, the seedling blight organism attacks comparatively few plants. In fact, it seems that correlations of a few factors with resistance and immunity in a single species have little bearing on the problem of specificity to attack observed in those same organisms. Attempted correlations of the reactions of the host to a fungus after penetration with resistance or susceptibility have likewise little to offer in a study on specificity to attack on those same hosts.

Another peculiarity of these host-parasite reactions which is often noted and just as often goes unexplained is the fact that very seldom do the hyphae of directly penetrating fungi enter stomata. All the facts accumulated thus far (except the actual observation of the non-entrance of stomata) would tend to show that hyphae of almost any fungus should enter stomata under many conditions. Actually, however, such does not seem to be true.

In other parts of this paper there is some discussion of the relative merits of the several techniques available for a study on specificity to penetration

by parasitic fungi. In no case can any technique be said to give "natural conditions." In that respect any investigation on specificity to attack by a fungus must be limited.

It should be emphasized that the present study is not wide enough in its scope to make generalizations concerning specificity to attack by fungi. Many other experimental hosts, a wide variety of both parasitic and strictly saprophytic fungi, and diverse environmental conditions must be employed before anything approaching a definite conclusion on this matter can be reached. As far as work reported in this paper is concerned, the only general statement to be made at present is that *C. circinans* appears to be parasitic, under certain conditions, on a number of species of plants.

This investigation indicates that the so-called specificity against attacking fungi is not generally due to any one of the following as a limiting factor —failure of spores to germinate, non-formation of appressoria, non-penetration, nor rapid death of the penetrating hyphae soon after entrance into the host. Work on the rusts of cereals, on *Erysiphe*, and to a limited extent on a number of directly penetrating fungi indicates that in resistance-susceptibility relations, the principal limiting factor is that of the death of the fungus soon after the host has been penetrated. It is doubtless entirely unsatisfactory to speculate at present as to whether or not the lethal effects (whatever be their causes) on fungous hyphae within resistant and immune strains of hosts is confined to resistance and immunity within a single species, or is also to be found as a limiting factor causing specificity against attack in a number of species.

A number of suggestions have been made as to the cause of the lethal effect on fungi in immune plants. I have already discussed these theories and have pointed out that it is difficult to believe that all species of plants except a few are so constituted that they are not the proper environment for a particular fungus. However, it must be admitted that if this argument is accepted without any reservations it will be extremely difficult to explain the causes of immunity and resistance on any grounds. It would seem that Walker is entirely correct when he says that any conclusions regarding resistance in a strain of plants must be based on facts gained from a study of that particular case. At present the same principle must be followed in a study of specificity to attack by fungi.

As regards the particular problem of the generally accepted specific host range of *C. circinans* in contrast to the results we have presented, indicating that under certain conditions this fungus can parasitize other plants, two pertinent questions might be asked. These questions are: Why does *C. circinans* not parasitize these plants under the normal cultural conditions of field and greenhouse? Under the conditions used in the present experiments, (*a*) why were some of plants penetrated oftener than others by the fungus; (*b*) why did one apparently not have viable penetration hyphae in it while the others did; and (*c*) why were some plants parasitized by *C. circinans* and others not?

Attempting an answer to the first question, it is suggested that one of the most important reasons for specificity in this case under "natural" circumstances is that environmental factors prevent (or at least do not aid) in the attack of the fungus on non-host plants. The conditions for the active parasitism by *C. circinans* on experimental hosts seem to be the presence of moisture at the right times, and possibly a heavy spore load in the inoculation drop. This suggestion is based on the following facts: (*a*) Moisture is necessary for the parasitism of *C. circinans* on onions; the recommended control measure for onion smudge is to keep the onions reasonably dry, rain during harvest being enough to afford the proper conditions for attack. (*b*) On the whole there is constantly more moisture in the soil than in the atmosphere; there are times when the air is saturated with moisture for quite prolonged periods, but often it is probable that such periods are at inopportune times for fungous attack. (*c*) The more spores of a fungus there are present the more likely is that fungus to infect its host plants (within limits), other conditions being equal; if enough spores of *C. circinans* are not present on the plants when conditions are correct, there is less likelihood that the fungus will infect them; seldom are large numbers of spores of *C. circinans* likely to come to rest on the above-ground parts of plants. The importance of the proper (and as yet unexplained) conditions is illustrated by this instance. A white onion bulb was inoculated with spores from a single culture of *C. circinans* in the usual way. There were large numbers of spores on the bulb, but in some places not one of those spores was germinated, while a few millimeters away practically every spore had germinated so that the bulb was almost solidly covered with appressoria in that area.

This reasoning does not in any way explain the situation as regards the obligate parasites; nor does it explain why roots in moist soil where *C. circinans* is present apparently are not parasitized under natural conditions.

A definite answer can not be given to the second question until more plants in any one of the species are studied. For example, it can not be definitely said that castor bean is specific in its resistance to attack by *C. circinans* until more castor bean plants are inoculated. That some specific compound conferring "immunity" is present in the case of the castor bean seems not to be a safe assumption at present.

Summary

1. A discussion of some of the concepts of resistance, immunity, and susceptibility as they might possibly relate to specificity to attack by disease inducing agencies is given.

2. Sections of leaves from 22 species of plants were microscopically examined. Twenty of the plants were penetrated by *C. circinans* under the conditions employed.

3. Indications are that the thickenings in the epidermal walls associated

with the presence of the appressoria are of similar natures, lima bean excepted. Ten species were tested microchemically.

4. Statistical studies revealed no definite indication that the reactions of leaves of various plants to *C. circinans* are narrowly specific. The reactions of 19 species were statistically classified.

5. Plating out experiments tend to show that some hyphae are still viable within the leaves of experimental hosts three days after inoculation. Previous to plating out, the leaves were surface sterilized. Of 20 species so studied, only one gave negative results.

6. Using an inoculation drop of 1: 10 prune juice, 16 varieties of plants showed lesions caused by *C. circinans*; 7 did not show lesions. Using an inoculation drop of water, 14 varieties of plants showed lesions; 9 did not show lesions. Using an inoculation drop of soil solution, 2 species of plants showed lesions; none showed no lesions. These results were obtained under certain conditions of moisture.

7. Attempts to secure a disease of peas caused by *C. circinans* by inoculating the soil in which the peas germinated and grew were unsuccessful. Inoculation of foilage of peas which were then kept under greenhouse conditions did not result in a disease of the peas.

8. Generally speaking, *C. circinans* seems to be more virulent in its parasitism when it has a saprophytic start, as in 1: 10 prune juice.

9. The evidence of microscopical examination, plating out experiments, and the fruiting of the fungus on living experimental hosts indicates that under certain conditions *C. circinans* is not specifically limited in its host range to some species of *Allium*.

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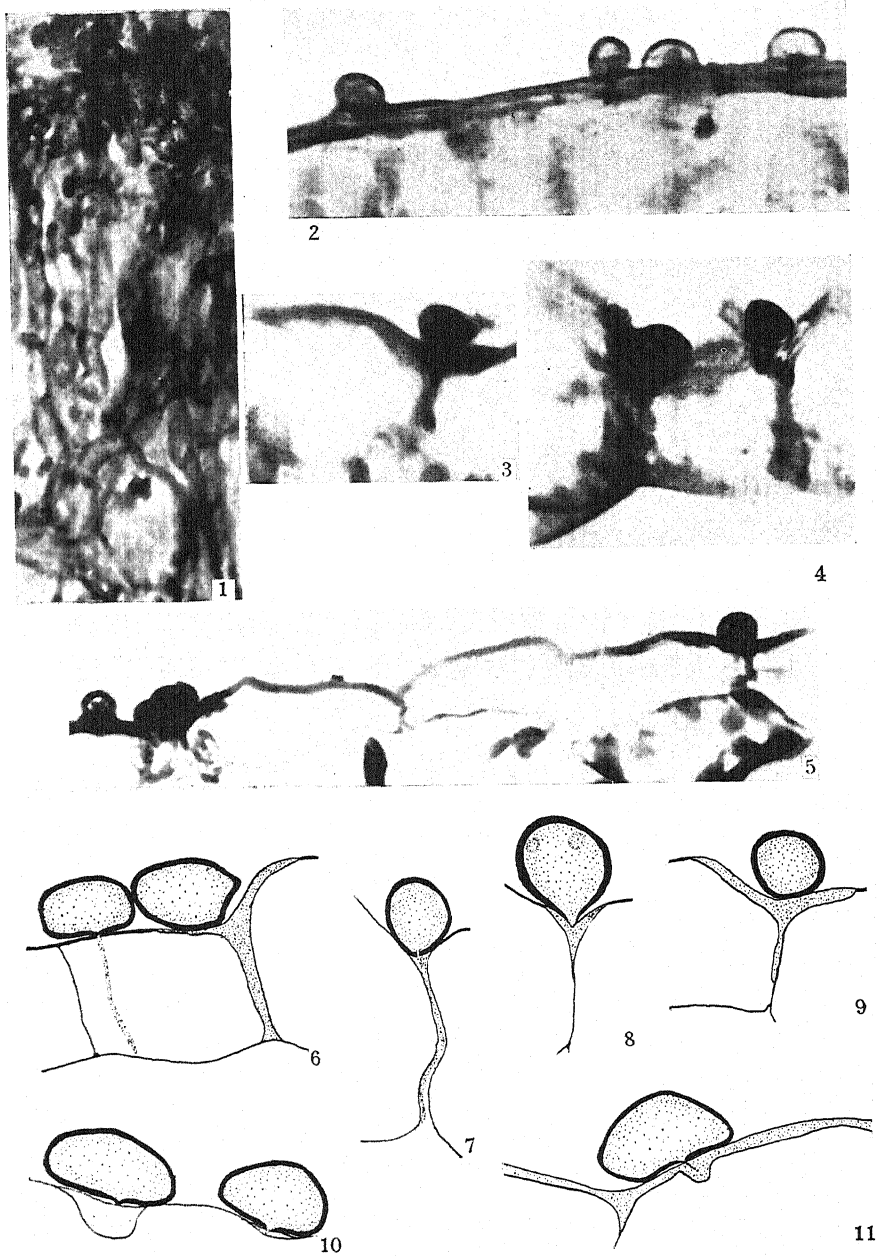
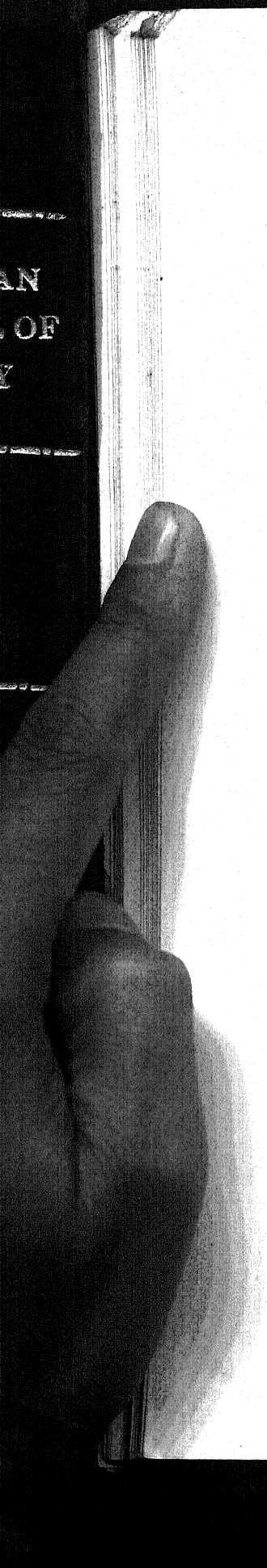


JOHNSON: SPECIFICITY TO FUNGUS ATTACK



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EXPLANATION OF PLATE I

FIG. 1. A small portion of a leaf of Dwarf Telephone pea. The tissue is practically disintegrated and hyphae are ramifying throughout the leaf. The upper part of the leaf covered with a mass of dark hyphae and appressoria is shown at the top of the photomicrograph.

FIG. 2. Soy bean. Penetrations through the collapsed upper epidermis of the leaf.
FIG. 3. Cotton. Reactions of wall of the upper epidermis.
FIG. 4. Buckwheat. Marked reactions of wall of the upper epidermis.
FIG. 5. Beet. Thickenings in the walls of the upper epidermal cells.
FIG. 6. Soy bean. Penetration and reaction in the walls.
FIG. 7. Bean. Possible penetration at the vertical cell walls of the upper epidermis.
FIG. 8. Mustard. A typical reaction for the walls of the epidermis of this host.
FIG. 9. Tomato. A typical reaction of the walls of the epidermal cells.
FIG. 10. Lima bean. Typical reactions of this plant to appressoria of *C. circinans*.
FIG. 11. Field pea. Minute penetration peg in a thickened epidermal cell wall.

THE MORPHOLOGY OF REGENERATION IN *BRYOPHYLLUM CALYCINUM*

ERNST NAYLOR

(Received for publication July 28, 1931)

Although a considerable amount of research has been done upon the physiology of regeneration in *Bryophyllum calycinum* Salisb., relatively little has been found concerning the histology and morphology of this phenomenon. Very few papers deal directly with the problem, and for the most part only casual references to it are made in the literature. Goebel (1) states that the shoots which appear at the notches of detached leaves arise in quite young leaves, appear at definite places, and belong to the normal course of development of the plant. Strasburger (2) says that, although meristems arise from the leaf, the development of these "buds" must properly be regarded as a part of the normal ontogeny of the plant, since they arise in predetermined positions from young tissue. Miss Beals (3) in a general histological study of regeneration in plants, reports that the meristematic tissue in the leaf notch is derived from the phloem of the vein. Miss Howe (4) in a recent publication indicates that the young plants originate from meristematic groups of cells which are present in young leaves and which are definitely organized into buds in the mature leaf. The following paper is the result of an investigation in which the morphology of the notch, as well as the early stages in the ontogeny of the leaf, were studied in considerable detail.

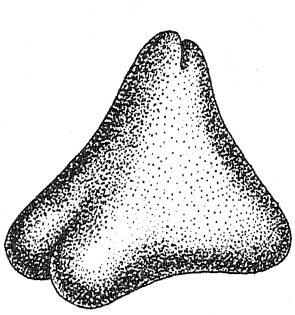
METHODS AND MATERIALS

Mature leaves were selected from rapidly growing plants, and small areas of the leaf tissue including the notches were cut out. These pieces were killed and fixed in a 1 percent solution of chromoacetic acid, carried through the customary histological technique, cut ten microns in thickness, and mounted serially. Sections of each notch region were made in either a horizontal direction, parallel to the leaf surface, or in a vertical direction, at right angles to the leaf surface. Vertical sections made at right angles to the edge of the leaf are called transverse vertical sections, and those made parallel with the margin of the leaf are termed tangential vertical sections.

For the study of very early stages in the formation of the leaf and the leaf notch the terminal buds of young plants were removed, subjected to the same technique of killing and fixing as employed for the mature leaves, and sectioned in the longitudinal and transverse directions. Safranin and Delafield's haematoxylin were used as stains.

Morphology of the Leaf Notch

It has been found from this study that the plant which grows from the mature leaf notch originates from a preformed structure which has existed in a dormant condition since early stages in the formation of the leaf. When the mature leaf is severed from the plant, this structure resumes activity; and entire young plants are soon differentiated. The preformed structure present in each notch is composed of a rudimentary stem tip, two root tip primordia, two leaf primordia, and a region which the author has named a *foot* because of its resemblance to that of the embryo of the fern and of other plants. This foot extends a short distance into the mother leaf and lies in contact with a vein. The presence of recognizable primordia of the essential organs of the entire plant indicates that a whole embryonic plant rather than a "bud," as indicated by Miss Howe, exists in each notch; and the word "embryo" is used for this body throughout the description which follows. The general external appearance of this embryo is illus-



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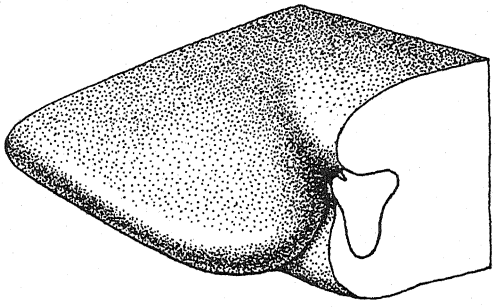

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Text Fig. 1. Diagrammatic drawing showing the external appearance of the entire embryo removed from the notch. Side view. × 100.

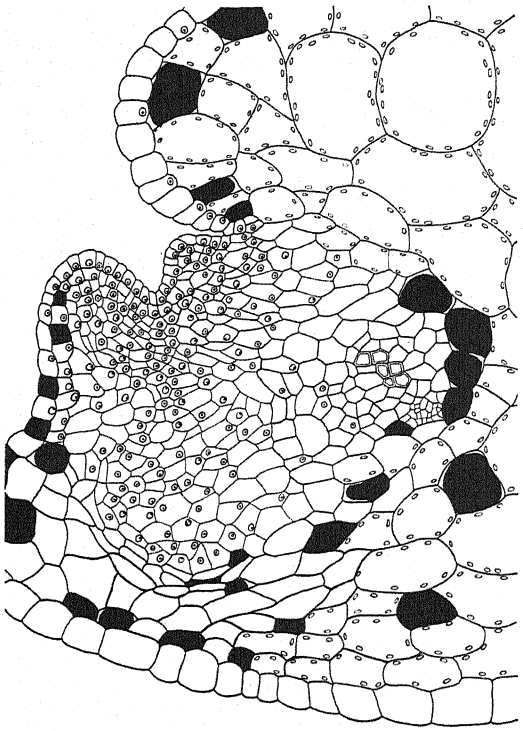
Text Fig. 2. Diagrammatic drawing of the notch of the leaf cut in a median transverse vertical plane showing the orientation of the embryo. × 30.

trated by text figure 1, which shows diagrammatically the two rudimentary leaves above, the two root primordia below, and the foot region to the right. The stem tip is located between the two leaves and is not visible in the sketch.

In the leaf notch is an indentation or fold parallel to the leaf surface and about halfway between the upper and lower surfaces. The two leaf primordia project slightly from the lower side of this cavity, and the remainder of the embryo is embedded within the tissues of the mother leaf. Text figure 2 illustrates the general orientation of the embryo when the notch is cut in the transverse vertical plane. The two root tip primordia are found almost directly below the leaf primordia, and in many instances the lower side of the leaf bulges out slightly around one of the root tips and becomes quite evident as soon as further development is resumed. The foot region

lies between the root and stem primordia and extends away from the notch and toward the interior of the leaf. It always terminates at a vein which is parallel to the margin of the leaf and which is joined on either side of the notch by veins extending from the interior of the leaf.

The detailed structure of the notch region and the relations of one part of it to another are best understood from a transverse vertical section because of the vertical orientation of the various parts. Text figure 3 illus-

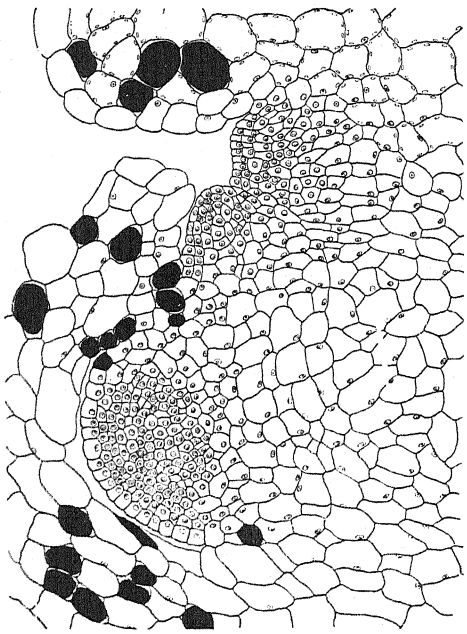


TEXT FIG. 3. Transverse vertical section of the dormant notch passing through the center of the embryo. The two leaf primordia shown above, one of the root tips below, and the vein to the right.

trates such a section. The most prominent structures are the primordia of the first two leaves. They bulge out slightly into the notch, and the primordium of the one leaf standing furthest out in the notch is always somewhat larger than the other which is nearest the mother leaf. Miss Howe remarks that one but seldom finds this protuberance slightly lobed. The cells of these primordia are small and stain more deeply than any other cells of the notch region except those of the root tip primordia. A rather distinct epidermal layer is formed early in the young leaves and is continuous with the epidermis of the mother leaf. The shaded cells scattered about through the figure are tannin-bearing cells, which are quite numerous near the epidermal layers and along the veins.

The stem tip primordium is not well illustrated because the section is not a perfectly median one. A short flattened region which represents the stem tip lies between the two leaf primordia. This stem tip is similar to that of the terminal bud of a mature plant which always remains covered with rudimentary leaves.

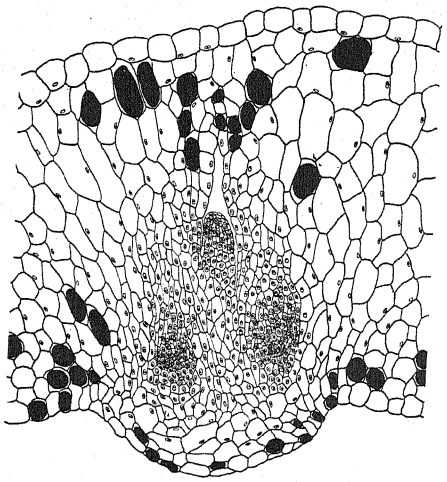
In general, two root tip primordia can be found opposite the leaf primordia and not far from the lower epidermis of the mother leaf. Since they do not lie in the same plane as the leaf primordia, only one root tip is visible in a transverse vertical section. In some embryos both primordia are very evident, whereas in others only one can be clearly demonstrated. The detailed structure of the notch appears to vary somewhat in individual leaves and no doubt in the notches of the same leaf. Neither of the root tip primordia extends straight downward, but they incline slightly away from the center of the leaf. Text figure 4 shows a median section of the root tip re-



TEXT FIG. 4. Transverse vertical section of the notch passing through one of the root primordia. Not median for the leaf primordia.

gion cut in a transverse vertical plane. It can be seen that the roots do not have their origin in "certain phloem cells," as stated by Miss Beals, or even from cells near the vein, as indicated by Miss Howe. They originate from small groups of cells on the lower side of the embryo and quite as far removed from the vein as are the leaves. Unfortunately the vein does not show in figure 4 but can be seen in figure 3, and the section is not median for the leaf primordia.

The small cells of the notch extend inward a short distance to a vascular bundle as shown in text figure 3. This bundle is a very constant structure and always marks the inward limits of the thin-walled cells. The xylem of the bundle consists mostly of reticulate tracheids, and the phloem is made up of elongated thin-walled cells which are very small in cross section. The orientation of the xylem and phloem here differs somewhat from that found in the bundles between the notches or elsewhere in the leaf. Usually the xylem is found directly above the phloem or toward the upper side of the leaf, but here it is found slightly to one side and always toward the notch.



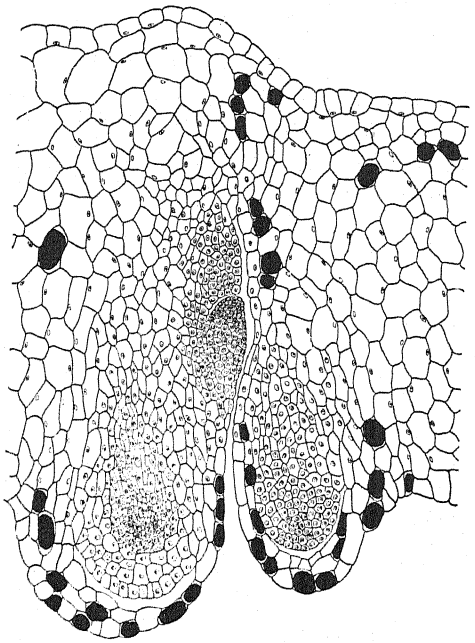
TEXT FIG. 5. Tangential vertical section through the dormant notch showing the two root tip primordia and one leaf primordium.

All the cells making up the embryo are sharply distinguished from the other cells of the leaf by their structure, contents, and staining capacity. The boundary of the embryo is fairly sharp and smooth. It extends from the two leaf primordia downward to within several layers of cells of the lower epidermis of the mother leaf and toward the interior a short distance to the vascular bundle already mentioned. Beyond these boundaries the ordinary large cells of the mesophyll are to be found.

Text figure 5 shows the dormant notch cut in the tangential vertical plane. In this plane only one of the leaf primordia can be seen bulging up into the notch. The embryo region becomes broader toward the lower side of the leaf and includes the two root tip primordia. The vascular bundle, which was visible in previously described sections, does not show here because the section is made too near the margin of the leaf to include part of this structure.

Text figure 6 represents a section similar to the preceding one, but made on the second day after the mother leaf was detached from the plant. Cell

division is evident in the regions of the root tips which are now pushing through the lower epidermis of the leaf. The root at the left appears slightly more advanced than does the one at the right, and from hundreds of sections studied it appears to be generally true that one root emerges a short time before the other. Neither of the root tips is cut in a median longitudinal



TEXT FIG. 6. Tangential vertical section through the notch on the second day after the leaf was removed from the plant, showing both root tips about to emerge.

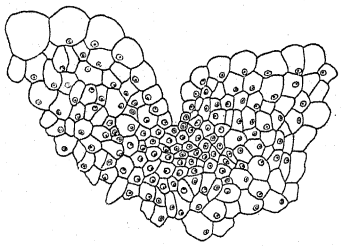
direction because of the slight angle at which they emerge. The roots push their way out through the cells on the lower side of the leaf in a manner similar to that in which secondary roots emerge through the cortex cells of the primary root. A narrow space not occupied by cells immediately in front of each root is commonly found in all sections. This is probably produced by shrinkage during the killing and fixing processes. There is an evident increase in thickness of the mother leaf at the notch region caused by the growth of the embryo before the roots and leaves make their external appearance.

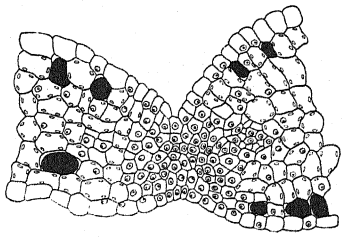
ORIGIN OF THE LEAF NOTCH AND EMBRYO

The investigation concerning the ontogeny of the leaf as a whole has shown that the notches are formed in the very early stages of development when the entire leaf is only a few millimeters in length. It has also shown that the embryo found in the notch of the mature leaf has its origin in a small group of cells along the margin of the embryonic leaf.

Sections through the terminal bud region demonstrate that the leaves have their origin as lateral projections of the embryonic stem tip. The initiating cells of the leaves have not been accurately determined, but apparently more than a single outer layer of cells is involved in the formation of this meristematic protuberance.

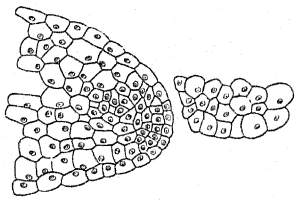
The notches along the margin of the leaf become evident at about the

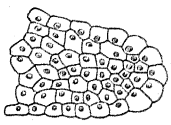




TEXT FIG. 7 (left). Horizontal section of the margin of the young leaf when only two or three millimeters long, showing the earliest stage in the formation of the notch.
TEXT FIG. 8 (right). Tangential vertical section of the very early notch.

time when the leaf is first noticeable to the naked eye or when it is between five and six millimeters in length. At this time the whole leaf is still embryonic, and both cell division and cell enlargement are taking place simultaneously. However, cell enlargement occurs more quickly in some parts of the margin than in others and causes these regions to bulge. The spaces between these bulges become indentations or notches and are occupied by cells which are still dividing but are not increasing appreciably in size. This observation is illustrated in text figure 7. The protuberances become more



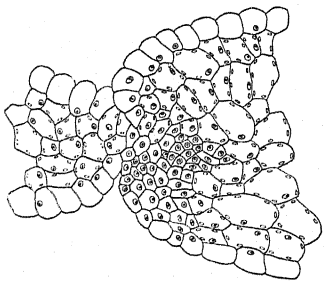


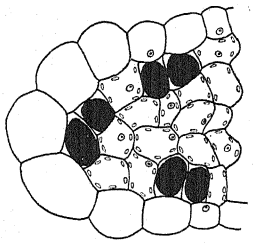
TEXT FIG. 9 (left). Transverse vertical section of the very young notch. Leaf approximately four millimeters in length.
TEXT FIG. 10 (right). Transverse vertical section of the margin of the young leaf between the notches. Age the same as in figure 9.

pronounced as the cells continue to enlarge, and small groups of embryonic cells are left in the notches and finally become surrounded by mature cells. Division continues for some time among these cells, with little increase in cell size until the young embryo is formed. Cell division then apparently ceases and this region becomes dormant in the mature leaf. Groups of cells

behaving in this manner along the entire margin of the young leaf form the series of notches.

Text figure 7 shows one of the earliest stages in the formation of the notch in a section parallel to the flat surface of the leaf. The general enlargement of the cells on both sides of the notch is apparent, especially in the epidermal cells. A similar situation is seen in text figure 8 in a section made in a tangential vertical plane. Text figure 9 illustrates the structure of the very early notch when cut in the transverse vertical direction. Text figure 10 is

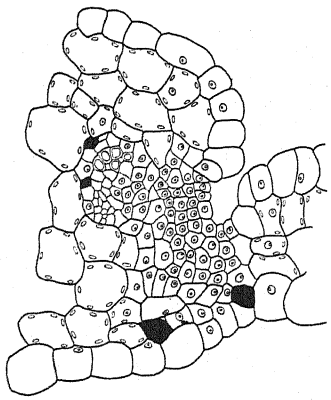




TEXT FIG. 11 (left). Transverse vertical section through the notch of a leaf slightly older than shown in figure 9. Leaf six to eight millimeters long.

TEXT FIG. 12 (right). Transverse vertical section of the margin of the leaf made between the notches. Age the same as in figure 11.

made in the same plane and through the margin of the same leaf as is figure 9, but located between the notches. The same regions described in the two previous figures are seen again in text figures 11 and 12 in a slightly older



TEXT FIG. 13. Transverse vertical section of a considerably older notch. Leaf about one centimeter long.

leaf between six and eight millimeters in length. The cells occupying the notch are now noticeable because of their size and contents. The ventral [1]

[1] The terms *ventral* and *dorsal* are used in the customary sense: the ventral side is the side next to the axis and the dorsal side that away from the axis.

side of the leaf is beginning to bulge out into the notch because of the more rapid increase in the size of the cells of this region. The vein has not yet differentiated.

Text figure 13 is a transverse vertical section through a notch of a leaf about one centimeter in length. The notch region now begins to resemble that of the mature leaf. A group of very small cells which is not yet organized as a recognizable primordium, is evident in the notch. The vein has been differentiated and shows the normal orientation of xylem and phloem. Later the vein becomes twisted so that the xylem is inclined toward the notch as shown in text figure 3. This is caused by the continued increase in the size of the cells toward the ventral side of the leaf without a corresponding increase in the total volume occupied by the embryonic cells in the notch.

From these observations it seems clear that the embryos present in the notches of the *Bryophyllum* leaf originate from small groups of cells along the margin when all cells are embryonic and are first evident when the leaf is only a few millimeters in length. These embryonic cells in the notches divide, form the root, stem, and leaf primordia of the young plant, and later become isolated by the maturation of the other cells of the leaf. When the whole leaf reaches maturity, cell division ceases in these regions and the embryos become dormant. When the leaf is detached from the plant, conditions are again made favorable for further activity; and the young plants make their appearance within a few days. The exact stage of development at which cell division ceases in these various primordia has not been accurately determined.

Summary

1. The primordia of entire new plants are already present in the notches of the mature *Bryophyllum* leaf.

2. The new plants originate from cells along the margin of the mother leaf when the latter is still in an embryonic condition.

3. There is no evidence that any part of the new plant originates from phloem cells of the vein of the mother leaf.

The writer wishes to acknowledge helpful suggestions from Dr. H. W. Rickett during the progress of this investigation. Thanks are also due the Missouri Botanical Garden for the original *Bryophyllum* leaves.

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STUDIES IN THE CHYTRIDIALES VII. THE ORGANIZATION OF THE CHYTRID THALLUS

JOHN S. KARLING

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In previous studies (1930, 1931) I have discussed in general the structural differences in the thalli of the various chytrids which have come under my observation. These differences are quite definite and striking in many respects, and merit particular attention. It is, of course, premature and impossible in the present state of our knowledge of the chytrids to say how significant these characteristic differences will prove in phylogenetic studies; but, since the lack of conclusive evidence of sexuality and the simplicity of organization in this group have made it necessary to base classification primarily on the complexity of the vegetative thallus, it is worth while to focus attention on these matters of structural differentiation. The present analysis, except for the forms which I have studied, is based entirely on the data found in the literature at the present time. The majority of chytrid species are little or imperfectly known, and their relationships are in most cases obscure. The group as a whole has come to be a sort of dumping ground for relatively simple and more or less unicellular fungus parasites and saprophytes which do not appear to belong to any natural phylogenetic group. Hence, subsequent discoveries, the elimination of doubtful species, and the addition of new ones may prove this analysis altogether inadequate, but for the time being it will serve to direct particular attention to types of organization which are characteristic in certain groups as they are known today.

This treatment or discussion of thallus organization is not primarily concerned with the evolution and phylogeny of the chytrids, and I shall accordingly avoid as much as possible the use of the term "morphology" in dealing with structural similarities and differences of species within a group or family or with the separate groups themselves. Since the time of Darwin and the origin of the doctrine of organic evolution morphology has meant not the study of the form and structure of organisms alone, but of this form as it has come about by descent, genetic relationship, or evolution, an implication which many authors and text-books have ignored. It is at once obvious to students of the chytrids that the evidence for the origin of the more complex forms from the simple types like members of the Olpidiaceae and Synchytriaceae is by no means conclusive; and in cases like the parasitic slime moulds and so-called Myxochytridineae, where structure and ontogeny are strikingly alike, this similarity may not necessarily mean genetic relationship but merely parallelism in development. Thus, to speak of the morphol-

ogy of the chytrids as a whole or of individual groups would be assuming in a certain sense a phylogenetic relationship for which we have no adequate evidence at the present time. The term "anatomy" is free from any implication of genetic relationship, and I shall use it wherever possible in contrasting the differentiations of the chytrid thallus.

The organization of the chytrid thallus is expressed in structural differentiations which are not clearly recognized by all authors. It is thus essential for the sake of clarity to indicate at the outset the sense in which many of the morphological terms in general use for these differentiations will be employed in this paper. I shall regard the thallus as constituting the entire fungus or algal plant which may become directly and physiologically transformed into a reproductive structure, or differentiated anatomically into fertile and vegetative portions at maturity. In this sense sporangia, resting spores, rhizoids, haustoria, etc. are as much a part of the thallus as the remaining less differentiated portions. Rhizoids, haustoria, holdfasts and appressoria are specialized parts of the thallus which have become structurally adapted to perform specific functions. It is evident from the literature, however, that there is little agreement as to the exact differences between them.

Although haustoria and appressoria had been seen and imperfectly described by Zanardini (1853), Von Mohl (1852, 1853) and others, DeBary (1870) was the first to devote intensive study to these structures. He applied the term *haustoria* to the structures as a whole, but failed to differentiate between the exterior anchoring and the real intra-cellular food-absorbing organs. Ten years later (1880) Frank gave the name *appressoria* to Von Mohl's *Haftorgane* and retained the term haustoria for the organs of absorption. Thus, very early appressoria were designated as non-penetrating anchoring organs. Smith (1900) has shown that in species of *Phyllactinia* on *Xanthoxylum americanum* where intercellular hyphae are abundant, appressoria are developed within the host tissue, but it is obvious from his figures that they are not intracellular. Frank's characterization of appressoria as non-penetrating organs for attachment must thus be extended to include the anchoring discs or sucker-like structures on intercellular hyphae.

As to the term *rhizoid*, it is used in the literature very loosely, in a general sense, for most filamentous and root-like structures of the fungus and algal thallus. In algae such as *Stigeoclonium*, *Penecillus*, *Caulerpa*, etc., filamentous but rather coarsely branched extensions occur at the base and at various intervals on the thallus which are anatomically very similar in many respects to the rhizoids of fungi and are described as such in the literature by Reinke, Børgesen, Svedelius, Woronin, Oltmanns and others. It is to be noted, however, that in the majority of algae possessing them these extensions are usually flattened, disc-shaped, bluntly lobed, or coarsely branched and bear little resemblance to a filamentous, thread-like, and finely divided rhizoidal system. Furthermore, in such autotrophic algae it is clear that they play

no important rôle in food absorption, and their function is primarily anchorage.

In the partially and completely extramatrical parasitic chytrids such as *Rhizidiomyces apophysatus*, *Rhizidium carpophilum*, *Phlyctidium*, *Phlyctochytrium*, *Polyphagus Euglenae*, *Sporophlyctis*, etc. the feeding organs are filamentous and finely branched, and taper to marked fineness at their extremities, and with the exception of size and extent of distribution are anatomically similar to the rhizoids of the more saprophytic forms like *Entophlyctis* and *Diplophlyctis*. Similarly in many saprophytic higher fungi, *Rhizopus nigricans*, *Mucor Mucedo*, *Araiospora pulchra*, *Rhipidium americanum*, *Blastocladia Pringsheimii*, etc., filamentous, root-like, branched structures are present which are almost universally described as rhizoids.

It is obvious from this that the term rhizoid has a wide application in the literature on thallophytes and has been extended to: (1) food absorbing organs in saprophytes, (2) food absorbing organs in parasitic chytrids, and (3) holdfasts or anchoring organs in certain algae.

As to the distinction between rhizoids and haustoria, the general tendency at present is to differentiate them as the feeding organs of saprophytes and parasites, respectively. This tendency is based to a large extent on the supposed differences in physiological relationship between host and pathogen and not primarily on sharp and well defined anatomical or structural differences, although as will become evident shortly such differences do exist. The body of a rhizidiaceous chytrid and the mycelium of a higher fungus are in general strikingly different in shape and extent of distribution. The chytrid body is usually spherical, oval, or somewhat broadly cylindrical and quite compact, while the mycelium is filamentous, diffuse, branched, and as a consequence widely extended in surface. Correlated to a certain extent with this body difference is another difference in the type of feeding organ. In the globular chytrids of the higher Rhizidiaceae like *Diplophlyctis* and *Polyphagus*, for instance, the food absorbing structures are filamentous, finely branched and widely extended and make up a well developed rhizoidal system. In the filamentous parasitic fungi such as the *Uredineae*, *Erysiphaceae*, and *Microthyriaceae*, on the other hand, where the thallus body or mycelium itself extends over a wide surface the feeding organs at one particular point or region are correspondingly reduced in extent of distribution and complexity of structures. In these forms the feeding organs or haustoria are predominantly sac- and club-like, but may become bluntly lobed, spiral, digitate and coralloid as Arnaud (1918) has classified them. It is obvious, however, from the drawings of Smith (1900), Arnaud (1918), Rice (1927) and others that even in these cases the haustoria bear little or no resemblance to the delicate thread-like extensions of the massive globular and ovoid chytrid body. Anatomically or structurally, then, there are in general certain differences between rhizoids and haustoria which in many cases go hand in hand with body type.

On the basis of host-parasite relationship there is clear evidence in the literature of a marked difference between these structures in many cases. In parasitic fungi like the powdery mildews and rusts noted above where sac-like, branched, and lobed haustoria are formed, parasitism is more or less of a symbiotic type; the parasite derives its food from the host cells without causing immediate fatal injury. There is no doubt that the fungus grows at the expense of the host, but with a regulated or "controlled mutualism." It is evident in many cases that the haustoria do not always come into direct contact with the host protoplasm or kill it prior to absorption. Smith (1900), Colley (1918) and others have shown that in many mildews and rusts the haustoria are separated from the host protoplasm by a definite delimiting sheath, so that the host cytoplasm makes contact only with the sheath membrane. Smith regards this membrane as possessing the same semi-permeable and osmotic properties as the plasma membrane of the cell, indicating perhaps an "extreme specialization in food preferences" by the fungus. In other cases (Allen, 1923; Rice, 1927) the haustoria invaginate the plasma membrane and cytoplasm of the host cell and thus often form so-called digestion cavities into which enzyms of the fungus supposedly pass.

As a consequence of this high degree of parasitic specialization and more or less balanced relationship between pathogen and host, infected cells with haustoria may have a healthy appearance with plastids, cytoplasm, and nuclei which for a time appear quite normal.

On the other hand, fungus parasites whose highly branched and extensive mycelium and rhizoids are frequently intra-cellular generally kill or injure the host tissue in advance and then feed on the dying and dead protoplasm. *Pythium*, *Phytophthora*, and *Sclerotinia* are notable and familiar examples of such relationship. In this same category, doubtless, belong the partially and completely extramatrical parasitic chytrids such as *Rhizidiomyces*, *Rhizidium*, *Polyphagus*, *Nowakowskia*, *Sporophlyctis*, *Rhizophlyctis*, and others. While the host-parasite relationship in these forms has by no means been as intensively studied as in fungi with haustoria, it is quite clear in *Polyphagus Euglenae* as well as in *Zoöphagus insidians* and *Sommerstorfia spinosa* of the Saprolegniaceae, at least, the rhizoids paralyze at once and kill the host cells in the same general manner as the nematocysts or stinging cells of *Hydra*, and then gradually absorb their contents.

In view of the structural as well as the physiological differences which have been briefly enumerated, I shall use the term rhizoid for any root-like, filamentous, finely branched and tapering extensions of the thallus which serve as feeding organs in saprophytes and certain parasites. The term haustoria will be limited to intracellular, sac- and club-like, bluntly lobed, coarsely branched or coralloid feeding organs of parasites which do not immediately injure or kill the host cell but live in a somewhat balanced relationship with it. Under the term holdfasts I shall include all flattened and disc-shaped, bluntly lobed, or branched and even coarsely filamentous

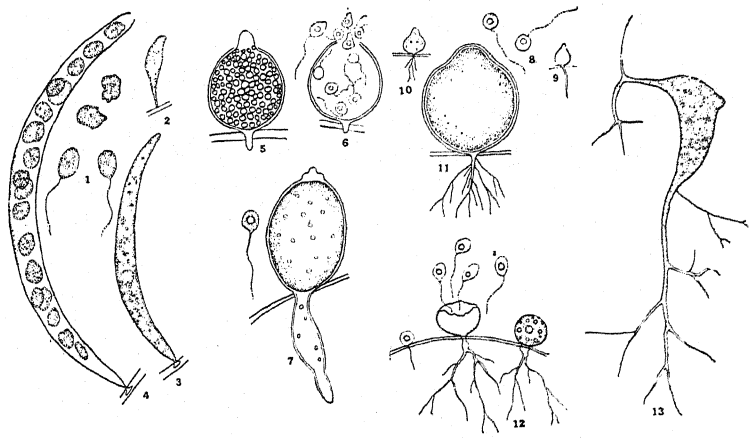
thallus extensions whose primary function is anchorage or attachment. Appressoria are thus included in this class as superficial or intercellular non-penetrating holdfasts. In this same category are placed the hyphopodia, stigmatopodia, and stommatopodia of *Balladyna*, *Meliola*, and *Paradiopsis* described by Gaillard (1892), Bucholtz (1897), and Arnaud (1918, 1921).

On the basis of thallus differentiation the chytrids fall into two fairly sharply delimited groups. In one group the thallus is naked at first and wholly undifferentiated, while in the second it is differentiated into fertile and vegetative portions in the form of zoösporangia, resting spores, rhizoids, and what I have called rhizomycelium. The first division includes the Olpidiaceae, Woroninaceae, and Synchytriaceae to which Gobi (1884) and Fischer (1892) applied the name Myxochytridineae; and in the second are placed the Rhizidiaceae, Cladochytriaceae, and the doubtful Hyphochytriaceae and Oöchytriaceae. There is a tendency on the part of several present-day mycologists, Gäumann (1926), Gäumann and Dodge (1928), and Fitzpatrick (1930), to include the parasitic slime moulds, Plasmodiophorales, in the former group also. Gäumann uses the name Archimycetes for this group as a whole, separates it entirely from the Chytridiales, and places it on an equal basis with the Phycomycetes, Ascomycetes, and Basidiomycetes. The term Archimycetes was originally proposed by Fischer for all of the chytrids as the first division of the Phycomycetes. Gäumann's first class of fungi is accordingly limited to Fischer's Myxochytridineae and the Plasmodiophorales. Fitzpatrick similarly includes the parasitic slime moulds with the chytrids, while Gwynne-Vaughan and Barnes (1927), on the other hand, exclude them and use the name Archimycetes for the combined Chytridiales, Ancylistales, and Protomycetales. The grounds for these differences of judgment are not very clear. Gäumann believes that the primitive and naked vegetative thallus, the lack of differentiation into fertile and non-fertile portions, and the close relation of this group to the Flagellata and Myxomycetes delimit it sharply from the remaining fungi. Gwynne-Vaughan and Barnes consider the lack of a mycelium or the presence of a rudimentary one evidence for including in the Achimycetes the most primitive fungi.

Rhizidiaceae

The thallus of this family consists of a cylindrical, oval or globular portion which is shortly transformed into a zoösporangium or resting spore, and a simple or highly developed and branched system of disc- and plate-shaped, tubular and root-like extensions. These may be reduced to mere holdfasts and simple haustoria in some species, while in others they are filamentous, highly branched, and widely extended and constitute an extensive rhizoidal system. With the view of presenting the structure and organization of the thalli in the Rhizidiaceae more concretely I have copied in text-figures 1 to 21 the drawings of some representative species as they have been given by various authors.

In text figures 1 to 13 are shown the thalli of *Harpochytrium*, *Phlyctidium*, *Chytridium*, *Rhizidium*, and *Rhizophlyctis* as they have been figured by Atkinson (1903, 1909), Braun (1855), Zopf (1885) and Petersen (1910). These figures show that the structural modifications at the base of the thallus vary widely as to size, shape, and extent of distribution. It is thus obvious that the thallus of these so-called simple members of the Rhizidiaceae shows



TEXT FIGS. 1–13. *Harpochytrium*, *Phlyctidium*, *Chytridium*, *Rhizidium*, and *Rhizophlyctis*. FIGS. 1–4, zoöspores, young and mature thalli of *Harpochytrium Hedenii* after Dangeard (1903) and Atkinson (1903). FIGS. 5 and 6, mature zoösporangium and escaping zoöspores of *Phlyctidium brevipes*, after Atkinson (1909). FIG. 7, mature zoösporangium and zoöspore of *Chytridium Olla*, after Braun (1855). FIGS. 8–11, zoöspores, young and mature thalli of *Rhizophidium globosum*, after Atkinson (1909). FIG. 12, zoöspores, young and mature sporangia of *Rhizidium carpophilum*, after Zopf (1885). FIG. 13, thallus of *Rhizophlyctis*, after Petersen (1910).

some differentiation, however it is to be interpreted. In *Harpochytrium*, a somewhat doubtful chytrid which Gobi (1899) placed among the Flagellates and Wille (1900) regarded as a reduced saprophytic alga, the thallus, according to the drawings of Gobi and Atkinson (1903), is attached to cells of various members of the Desmidiaceae by a flattened disc which does not penetrate into the lumen of the cell but is confined to the layers of the cell wall. Dangeard (1903), on the other hand, figures the disc inside of the cell and in direct contact with the protoplasm, and together with Lagerheim regards *Harpochytrium* as an active parasite which kills the host cell. Gobi and Atkinson question its parasitic nature. They have found the organism in great numbers on healthy cells, and in no case has the host cell been killed by it. Until further positive evidence is brought to light as to its parasitic nature, I shall regard the flattened disc-like organ at the base of the thallus as a holdfast structurally adapted for attachment and not primarily for food absorption. Fitzpatrick, however, speaks of it as an attenuated mycelium "which has been flattened at its tip to form a small disc which is either ap-

plied to or developed in the host wall." This is perhaps too liberal an interpretation of the word mycelium and robs it of much of its significance. In *Phlyctidium brevipes* Atk. (von Minden 1911–1915), text figures 5 and 6, this basal structure seems to be somewhat further developed, projects as a blunt peg into the lumen of the cell, and undoubtedly comes into direct association with the host protoplasm. Atkinson speaks of this structure alternately as a rhizoid and a penetration tube which serves both as a food absorbing organ and for attachment. In another species, *P. sphaerocarpum*, the peg is more pointed and appears almost filamentous and often penetrates as far as the center of the host cell. According to the descriptions and figures of Zopf and Atkinson (1909) there is no doubt as to the parasitic nature of this species and its lethal effect on the host. Atkinson claims that the initial effect is hypertrophic, an increase in length and size of the host cell, *Mougeotia*, and the size of the chromatophore. Following this the chromatophore and remainder of the protoplasm begin to degenerate and disintegrate and flow gradually towards the region where the chytrid is attached. Zopf, however, did not observe and figure hypertrophy and described only a gradual killing and degeneration of the host protoplasm.

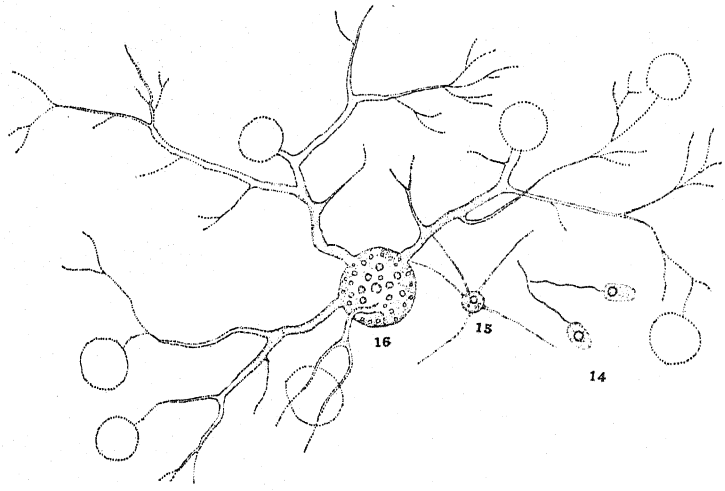
These two species of *Phlyctidium* thus show considerable variation in the size and shape of the structure at the base of the thallus and its effect on the host, according to the drawings and figures of Atkinson. From the anatomical as well as the physiological aspects the peg in *P. brevipes* is more like a simple, blunt haustorium which doubtless also functions as an organ for attachment; while in *P. sphaerocarpum*, where the effect on the host has been better figured and described, it shows perhaps a more rhizoid-like habit, although still unbranched.

In *Chytridium Olla*, text figure 7, the host and parasite relationship is not very well known. Braun believed it derived its nourishment from the organism, *Oedogonium*, which it parasitizes, but it does not kill the host cell or injure it to any marked degree, according to his figures and descriptions. If Braun's observations are correct, the relation between host and parasite is similar to that in the rusts and mildews described above where the immediate injury seems to be reduced to a minimum. In that case the organ at the base of the thallus may perhaps be regarded more as a haustorium. Anatomically it consists of a broad tube which extends for a considerable distance into the host cell.

The species so far considered have been almost wholly extramatrical except for the small disc- and peg-like thallus extension into the host cell. It is at once obvious, then, that there are varying degrees of extramatricality. It may vary in different genera and species from almost entirety to the condition where the thallus is wholly intramatrical. There appears to be little or no correlation between degree of extramatricality and parasitism. Completely extramatrical species like *Polyphagus Euglenae* may be highly parasitic and pathogenic or, as in *Harpochytrium*, saprophytic or at most

weakly parasitic. Similarly *Entophlyctis* and *Diplophlyctis* are completely intramatrical and largely saprophytic, while *Physoderma* and *Urophlyctis* are parasitic.

We may now consider some partially extramatrical species in which there is little doubt as to the presence of well defined, rather delicate, and more or less finely branched rhizoids. *Rhizophidium globosum*, *Rhizidium carpophilum*, *Phlyctochytrium quadricorne*, and *Rhizidiomyces apophysatus*, text figures 17 to 21 (Atkinson, Zopf, and Büsgen) may be taken as representative



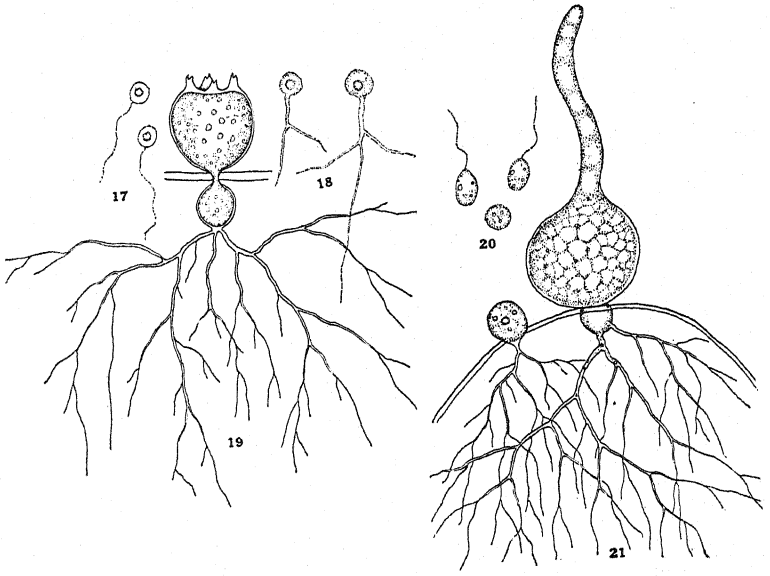
TEXT FIGS. 14–16. Zoöspores, young and more mature thalli of *Polyphagus Euglenae*, after Nowakowski (1877).

examples. In the first species, according to Atkinson's (1909) figures, the rhizoidal portion of the thallus is rather limited in size and extent of distribution in comparison with the zoösporangium. It appears to consist of a central axis from which grow out numerous fine branches which taper to a fine point. Atkinson figures the rhizoids in contact with the host protoplasm but says nothing of the parasitic nature of this species. Braun, however, describes it as injuring and killing filaments of *Oedogonium*, and it is perhaps probable that the rhizoid kills the host protoplasm before absorbing it. It must be borne in mind, however, that many of these chytrid species are minute relative to the size of the host cell, and in filaments which are parasitized the injury caused by a single thallus, although perhaps locally lethal, may not be sufficient to kill the entire cell or appreciably alter its appearance.

In *Rhizidium carpophilum*, parasitic on the oöspores of *Achlya*, the rhizoidal system is a bit coarser and more extensively distributed. Moreover, there is a tendency to develop a subsporangial swelling within the host cell from which the rhizoids appear to originate. This tendency reaches full expression in *Phlyctochytrium quadricorne* and *Rhizidiomyces apophysatus* in the formation of the so-called apophysis. The rhizoidal system of these two

latter species is more highly developed, branched, and extended, and often ramifies in the entire host cell, apparently killing the protoplasm first and absorbing it gradually. The whole system is centered on the apophysis and appears to have arisen from it, but as Zopf and I have shown, the rhizoids develop before the apophysis. Later as it increases in size and reaches maturity the rhizoids become centered on it rather than on the base of the zoösporangium.

The formation of the apophysis, it seems to me, is to be regarded as a tendency to homoeosis or reduplication. The incipient zoösporangium begins as a swelling of the penetration tube almost simultaneously with the formation of the rhizoid rudiments. The apophysis develops subsequently



TEXT FIGS. 17–21. *Phlyctochytrium* and *Rhizidiomyces*. FIGS. 17–19, zoöspores, germinating spores, and a mature thallus of *Phlyctochytrium quadricorne*, partly after Rosen (1887). FIGS. 20–21, zoöspores and young and mature thalli of *Rhizidiomyces apophysatus*, after Zopf (1885).

as an enlargement of the same structure between the incipient zoösporangium and the point of origin of the rhizoids. However, its growth ceases shortly, so that in no case so far described does it attain the size of a zoösporangium or give rise to zoöspores. Nonetheless, it is formed in a similar manner from the same anatomical structure of the developing thallus, and its formation is thus to be regarded as a tendency to homoeotic development, the formation of a like globular structure in this particular case.

In this respect the apophysis is quite different from the rhizoids whose formation is distinctly heterotic. I use the term heterosis here in its more general sense as meaning unlike in formation. There is a tendency at present, however, to use it in a more restricted sense as synonymous with

heterozygosis, the development or formation of a different, unlike constitution as a result of gametic union. The term heterozygosis is a very apt and appropriate descriptive word for such restricted cases of unlike formation, and should, doubtless, be retained in this limited sense. But to substitute it for heterosis in general would be excluding many types of unlike formation which are in no way zygotic. The formation of holdfasts, haustoria, and rhizoids in the chytrid species described above are thus typical cases of heterosis in its general sense. In a like manner the development and growth of the penetration tube is also heterotic. In germination and subsequent penetration of the host cell, the oval or globular chytrid zoöspore does not form a similar spherical structure but a fine, pointed, cylindrical filament which is markedly different anatomically in every respect.

It is to be particularly noted that in all of the extramatrical species so far considered the center of gravity of the growth process remains in the spore. In germination a filamentous penetration tube is formed which develops into a holdfast and haustorium, as in *Harpochytrium*, *Phlyctidium*, and *Chytridium*, or a rhizoidal system as in the other species described; while the zoöspore itself, by subsequent growth, enlargement, and differentiation, becomes transformed either into a zoösporangium or a resting spore. In this same category belong *Sporophlyctis* (Serbinov, 1907), *Rhizophlyctis* (Borzi, 1885), and *Polyphagus Euglenae*. The thallus of these genera is wholly extramatrical except for the tips of the rhizoids. Moreover, it is parasitic and polyphagous, attacking several host cells at one time. We thus have here another type of extramatricality in which the parasite derives its food from several host cells at the same time through a system of more or less radially oriented rhizoids. This tendency towards complete extramatrical development in these genera is accompanied by the loss of the penetration tube. According to the descriptions of Nowakowski and Borzi the zoöspores come to rest and send out several filamentous finely pointed rhizoids which eventually come into contact with and penetrate the host cells. As the thallus grows and matures the rhizoids generally branch several times so that an extensive more or less radiating rhizoidal system is established. The center of gravity, however, as in the previous genera, remains in the enlarged and differentiated zoöspore.

In *Polyphagus Euglenae* and *Sporophlyctis rostrata*, it is true, the differentiated zoöspore is not directly transformed into a zoösporangium but becomes the so-called prosporangium. The zoösporangium forms from this latter structure as a thin-membraned oval, elongated or cylindrical vesicle which shortly undergoes cleavage into zoöspores. However, the formation of this zoösporangium is not to be regarded, it seems to me, as a migration of the center of gravity of growth and differentiation in the same sense as it occurs in the following groups.

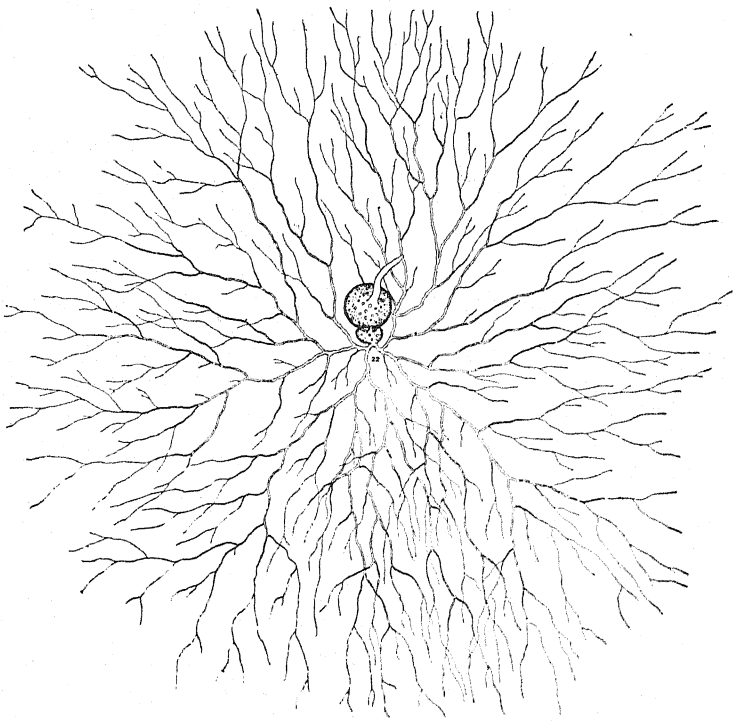
The development of the zoösporangium from the prosporangium is homoeotic. We see another instance of tendency in this direction in the

formation of the round or oval resting spore. According to Nowakowski, Dangeard (1900) and Wager (1913) a portion of the conjugation tube nearest the larger of the two fusing thalli enlarges considerably with the union of the two protoplasts and finally becomes the rough-walled resting oöspore. If the descriptions and figures of these authors are correct, sexual differentiation in *Polyphagus Euglenae* is homoeotic. The same is true of *Sporophlyctis rostrata* where the fusion, according to Serbinow (1907), is between individuals directly instead of through a conjugation tube.

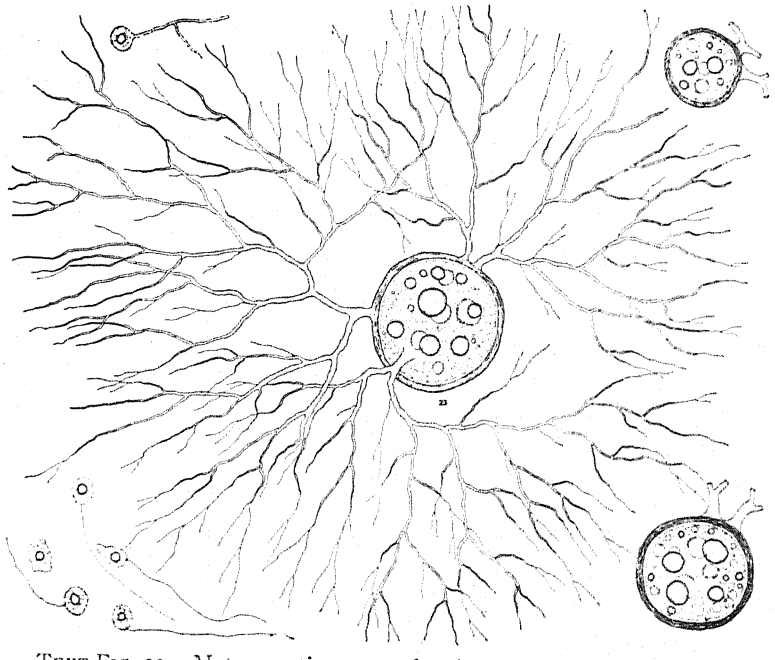
We may next consider the completely intramatrical species of the Rhizidiaceae in which the center of gravity is carried away from the zoöspore to some immediate outgrowth of it. *Entophlyctis* and *Diplophlyctis* are excellent illustrations. In these genera the germinating zoöspore forms a delicate penetration tube which pierces the wall of the host cell and differentiates almost at once into a rhizoidal and globular portion. The formation of the penetration tube and rhizoids is, as noted before, heterotic, while that of the globule is homoeotic. This latter part then becomes the center of gravity of growth and differentiation, and the zoöspore case and penetration tube soon disintegrate. Each zoöspore thus forms a single center or a monocentric thallus, and in this respect is unlike those of most of the Oömycetes which germinate into a mycelium or thallus in which are established with the coming of asexual and sexual maturity, secondary, tertiary, and quaternary centers in the form of sporangia, antheridia, oögonia, and oöspores, resulting thus in a polycentric thallus or system.

In text figures 22 and 23 are shown the thalli of a giant species of *Entophlyctis* and *Diplophlyctis intestina* as I have observed them in cells of *Nitella* and *Chara*. In these forms rhizoid development among chytrids reaches a maximum. The rhizoids of the species shown in text figure 23 may extend for a distance of 700 μ, and at maturity become very coarse, olivaceous in color, thick-walled, and bristle-like in appearance. Furthermore, at their point of origin on the zoösporangium or resting spore they may be as much as 6 μ in diameter, which is considerably greater than that of the mycelium of many of the higher fungi. Fineness and delicate structure are thus not necessarily the most outstanding characteristics of rhizoids. They soon begin to taper, however, and run out to almost invisible fineness at their extremities. In other species of *Entophlyctis* the rhizoids may be very fine, delicate, and limited in extent. Rhizoids may arise from a single point, generally opposite the position occupied by the zoöspore and penetration tube, on the surface of the zoösporangium or resting spore, or at several and various places, as is shown in text figure 23. In the former case when there is a single rhizoid the thallus is frequently described as monorhizoidal, but in the latter as polyrhizoidal.

In *Diplophlyctis intestina* the rhizoidal system is equally well developed and extended and perhaps more radially symmetrical in its distribution, as is shown in text figure 22. As a result the mature thallus has a superficial



TEXT FIG. 22. Mature thallus of *Diplophlyctis intestina*.



TEXT FIG. 23. Mature resting spore of a giant species of *Entophlyctis*.

resemblance to a large fungus spore which has germinated and sent out germ tubes in all directions. In large thalli the rhizoids may extend over a radius of 400 μ, but they are all, nonetheless, centered on the apophysis at the base of the zoösporangium.

A general survey of this nature brings out several conspicuous characteristics of the thallus of the Rhizidiaceae. First, that the rhizoids, as far as our present knowledge goes, decrease in diameter from the point of origin and run out to fine filaments *without any tendency to form new centers of growth and differentiation.* The same tendency is also exhibited by the rhizoids on the mycelium of *Piptocephalis* on *Mucor Mucedo*, *Rhizopus nigricans*, *Gonapodya*, *Blastocladia*, *Araiospora*, and *Rhipidium;* by the filamentous holdfasts of *Stigeoclonium*, *Penicillus*, *Caulerpa*, and *Bryopsis;* and also by the so-called rhizoids of the moss protonema, thallose liverworts, and fern prothalli. This constitutes one of the essential differences between rhizoids and the mycelium of the higher fungi, and justifies the distinctions which have been made above. Tapering of mycelial filaments is not, of course, uncommon at the edges of fungus colonies on culture media, but this is generally after the vegetative mycelium has established its anatomical indentity as a mature thallus. That tapering of the rhizoids is not an expression of thallus maturity in the Rhizidiaceae is shown by the fact that they nearly always run out to unusual fineness at their extremities even in the very early stages. The rhizoidal system is limited to the needs of the nutrition and development of a single sporangium; that is, the life cycle is limited to a single zoösporangium or resting spore.

Mycelial hyphae, on the other hand, soon carry the center of gravity to additional points where new centers of both asexual (sporangia, pycnidia, ascervuli) and sexual (oögonia, oöspores, zygospores) reproductive structures are formed. Growth and development in these forms thus become periodically vegetative and reproductive and thus may be said to show gradients to a certain extent. The movement of accumulated foods, at any rate, is not backwards to the spore.

It is for these reasons that the use of the word mycelium for the rhizoids of the Rhizidiaceae by many students seems unfortunate, unless, of course, it is employed as a purely anatomical term for all thread-like or filamentous structures. The arrangement of species, genera, and families of the Chytridiales by Gäumann, Gwynne-Vaughan and Barnes, Gäumann and Dodge, and Fitzpatrick, however, indicates that they regard the term in a morphological and phylogenetic sense, and implies that the mycelium of the higher fungi has evolved from the more complex type of rhizoids and rhizomycelium of the Rhizidiaceae and Cladochytriaceae, and hence that fungi are more or less monophyletic in origin. The hypothesis of a monophyletic development of the mycelium of all higher fungi through a continuous evolutionary series of rhizoids from the chytrids seems very doubtful in view of the structural differences between rhizoids and mycelia described above. The

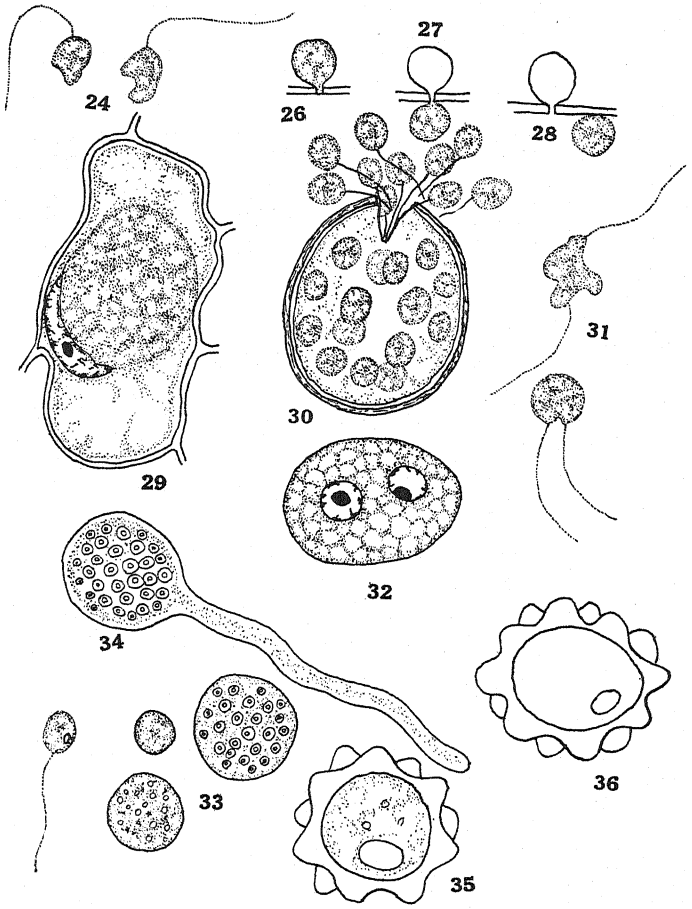
contrasting view that the mycelium is in many respects similar and morphologically equivalent to the filaments of the algae which have lost their chlorophyll, and has originated at several points among the algae, was perhaps first suggested by the studies of Pringsheim (1858). It culminated and reached final expression in the systems of classification proposed by Cohn (1872) and Sachs (1874). Sachs grouped the Chytridiales with the Protococcoideae, the Oömycetes with the Siphoneae, the Zygomycetes with the Conjugatae, and the Ascomycetes and Basidiomycetes with the red algae. These and other authors believed that the parasitic and saprophytic mode of life had occurred at various points among the algae by the loss of ability to form chlorophyll, thus giving rise to various groups of fungi. While the evidence for this view is not entirely convincing, it is not confronted with as difficult and serious problems as the monophyletic view.

The second outstanding characteristic brought out by this survey is that the thallus is unicellular up to the time of cleavage and definitely centralized. Since the center of gravity of the growth and differentiation processes is almost at once transformed into reproductive organs, zoösporangia and resting spores, the thallus of these chytrids may in general be spoken of as *reproductocentric* or *genocentric* in organization. The use of the prefix *geno* in this connection may not be in line with the present-day tendency to associate the term with sexual reproduction, but basically it should have a wider and more general application. This conception of genocentricity implies also that if the reproductive center produces only non-sexual zoöspores and resting spores the thallus organization is asexually genocentric, while if gametes are formed it is sexually genocentric.

It is also obvious from this consideration that each zoöspore, as noted before, produces a single center of growth and differentiation. This appears to be a universal characteristic of the Rhizidiaceae, and to my knowledge only two exceptions have been reported and figured in the literature (Fisch 1882, figures 11, 18, and 19; Zopf 1885, Pl. 17, fig. 17). Neither of these reports has been confirmed in subsequent studies. The thallus in this family is accordingly *monocentric*, and since the single center as has been described above shortly becomes a reproductive structure it follows thus that the thallus of the Rhizidiaceae at maturity is *monogenocentric*.

The thallus of the Rhizidiaceae is not only centralized morphologically but also to a great extent in movement of the accumulated food substances. In the germination of the zoöspore and the formation of the rudiments of the rhizoidal system and zoösporangium or resting spore, growth and the movement of accumulated food material is for a short time centrifugal or away from the spore, but once a center of growth and differentiation has been well established the movement is largely centripetal. In *Diplophlyctis intestina*, for instance, and other species whose ontogenetic development has been rather intensely studied, the incipient zoösporangium or resting spore remains relatively small until the rhizoids have developed, and then it

begins to enlarge quite rapidly. At this stage the movement of the accumulated food substances, aside from the small amount that may be used up in the further growth of the rhizoids, is undoubtedly backwards to the zoösporangium.

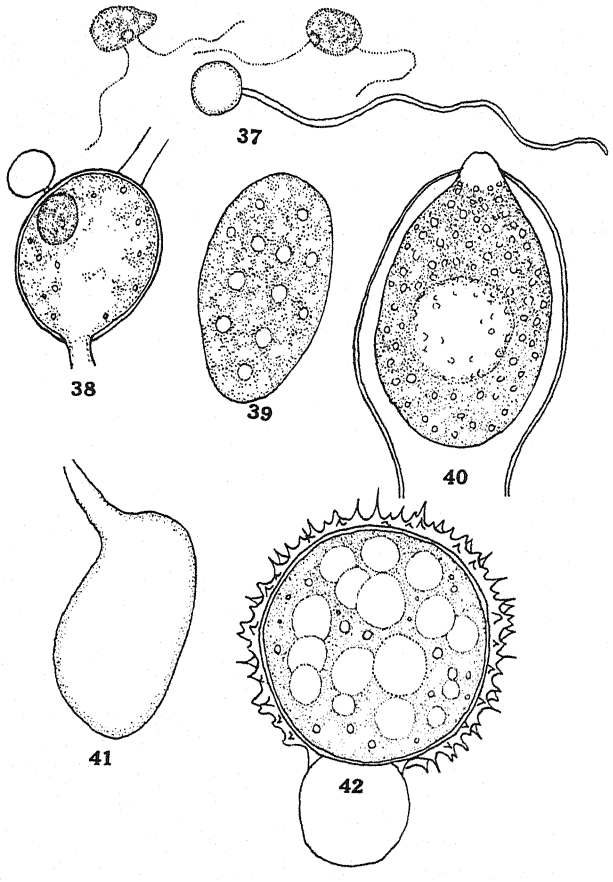


TEXT FIGS. 24–36. *Olpidium Viciae* and *O. Brassicae*. FIGS. 24–28, zoöspores of *O. Viciae*, and several stages in germination and penetration of the host cell. FIG. 29, a naked thallus in the host cell. FIG. 30, mature zoösporangium. FIGS. 31 and 32, fusion of zoöspores and a binucleated zygote, after Kusano (1912). FIGS. 33–34, zoöspore and stages in the development of the zoösporangium of *O. Brassicae*. FIGS. 35–36, resting spores, after Woronin (1878).

MYXOCHYTRIDINEAE

This same type of centric organization is also characteristic of the Olpidiaceae, Woroninaceae, and Synchytriaceae or the Myxochytridineae of Gobi and Fisher. In these families the thallus is not differentiated into fertile and vegetative portions, and lies more or less naked in the host cell like a plasmodium. In text figures 24 to 50 are shown the thalli of *Olpid-*

ium, *Olpidiopsis*, and *Synchytrium* as they have been figured by various authors. These forms are good representatives of the Myxochytridineae as a whole and may be taken as examples. Without going into a detailed description of the individual species, it may be said in general that in all of these forms the zoöspore penetrates the host cell and grows at its expense as a somewhat amoeboid or plasmodium-like body, completely devoid of rhizoids. At maturity the thallus is transformed wholly into zoösporangia,



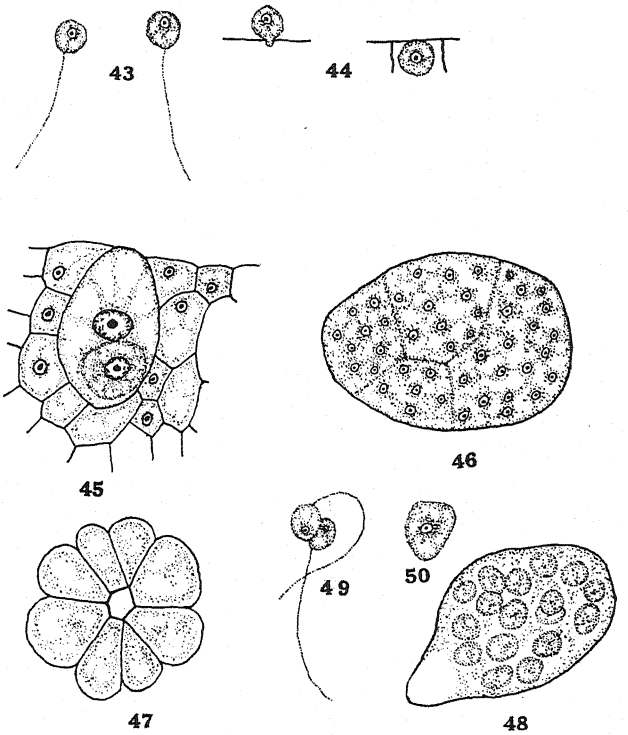
TEXT FIGS. 37–42. Zoöspores, germination of a spore, penetration of host cell, young and mature thalli in host cells, empty zoösporangium, and mature resting spore of *Olpidiopsis luxurians*, after Bartlett (1912).

resting spores, or groups of these in the form of sori, as in the Synchytriaceae, for instance. Whether or not the evidence of sexuality which Barrett (1912), Kusano (1912), Curtis (1921) and others have presented is convincing does not alter the situation as to the organization of the thallus. The center of gravity of metabolism, growth, and differentiation shortly becomes a reproductive structure, and in this respect the thallus is monogenocentric.

If the claims for sexuality in this group are confirmed and proven we have thus examples of both asexual and sexual monogenocentric thalli.

CLADOCHYTRIACEAE

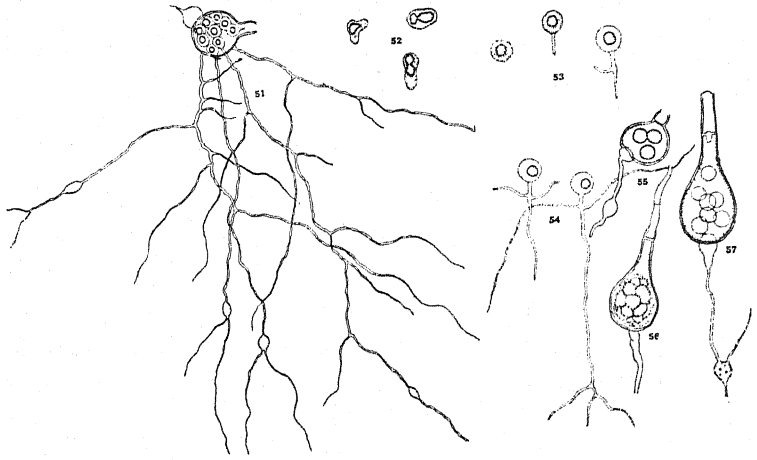
In the family Cladochytriaceae the thallus is more complex and presents a somewhat different type of organization. As I have noted before (1930, 1931) it is replicated or reduplicated to a certain extent as it spreads in the substrate on which it is growing or from one host cell to another and thus establishes successively new centers of growth and differentiation. The tendency to homoeosis is thus more pronounced. In this respect it is similar to the mycelium of the higher fungi, but for the sake of clarity in terminology I have designated the thallus as a rhizomycelium.



TEXT FIGS. 43–50. *Synchytrium endobioticum.* FIGS. 43–44, zoöspores and stages in the penetration of the host cell. FIG. 45, a hypertrophied host cell with the developing parasite thallus. FIG. 46, a young sorus undergoing cleavage. FIG. 47, rosette stage. FIG. 48, a mature zoösporangium. FIGS. 49–50, fusion of zoöspores to form the zygote, after Curtis (1921).

As in the case of the previous families there are presented in text figures the thalli of various genera and species as they have been figured in the literature. The knowledge of this group as a whole is as yet very incom-

plete, and there is little agreement as to the limits of the family. Furthermore, the lines of distinction and relationship between the different genera are not clear and sharp, and hence it is impossible to present a natural evolutionary series. We may begin with *Amoebochytrium* (text figs. 51–57) since it has been described as a relatively simple member of this group. Although the forms to be considered show varying degrees of thallus complexity, the order in which the following genera and species are presented is not to be taken as a natural and phylogenetic one. The thallus of *A. rhizidioides*, according to Zopf's (1885) description and figures, is a delicate extensively branched rhizoid-like system of filaments, and for this reason was at first placed between the Rhizidiaceae and Cladochytriaceae. The extremities of this system are very fine like rhizoids, and it possesses additional structures which are lacking in the former family—swellings or enlargements at various intervals on the rhizomycelium. These either become trans-



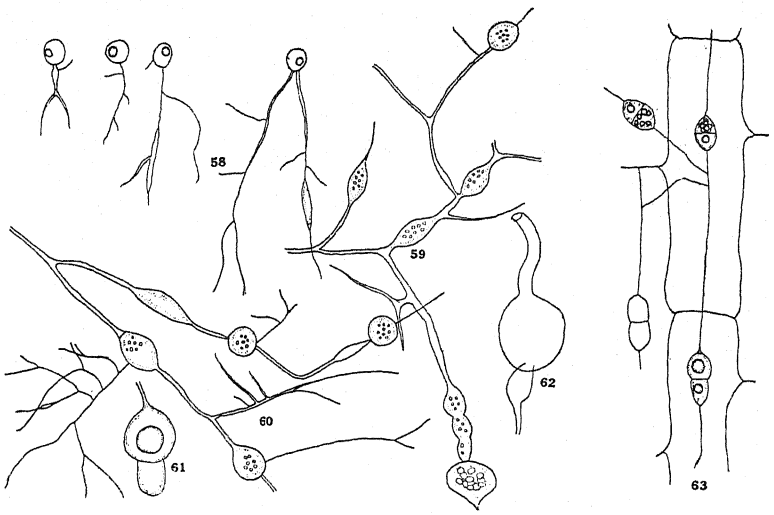
TEXT FIGS. 51–57. *Amoebochytrium rhizidioides*. FIG. 51, thallus of *Amoebochytrium rhizidioides* with several enlargements on the rhizomycelium. FIGS. 52–54, amoeboid zoöspores and germination stages. FIGS. 55–57, mature zoösporangia, after Zopf (1885).

formed into zoösporangia (text figs. 55 to 57) or remain only slightly modified in structure. Thus at maturity the thallus may have several centers in the form of zoösporangia.

Some of these enlargements may undergo division into two cells, one of which becomes the zoösporangium while the other remains unmodified and attached like an apophysis (text fig. 55). Others change very little except to increase slightly in size and remain continuous with the rhizomycelium. They are doubtless comparable to the turbinate organs of *Cladochytrium*, *Physoderma*, and *Urophlyctis*, and in view of the relation of these structures to the organization of the thallus I shall deal with them in detail in each genus.

These modifications of the vegetative thallus have long been known and

bear a large number of names. Büsgen (1887) noted their relation to the resting spores, and claimed that they accumulated food substances which were later used in the formation of additional "hyphae" and resting spores. Hence he gave them the name "Sammelzellen." Schröter (1889) regarded the individual cells as gametes whose contents fused to form the resting spore; and since in the genus *Urophlyctis* one of the cells remains rather small and frequently closely attached to the resting spore Magnus (1897, 1901, 1902) called them antheridia. They have also been named "corps centrales" by Cornu (1883), "Anhangzellen" by Fischer (1892), "vesicles collectives" by Vuillemin (1897), "turbinate cells" by Jones and Dreschler (1920), "collecting cells" by Bartlett (1926), and "turbinate organs" by



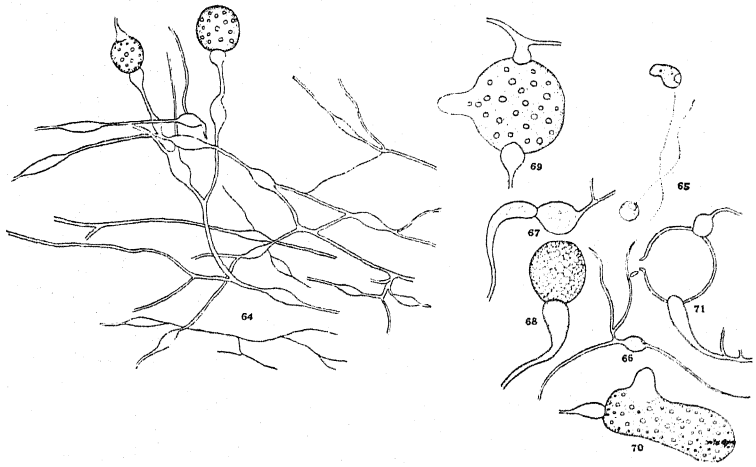
TEXT FIGS. 58–63. *Cladochytrium tenue.* FIG. 58, germination stages of zoöspores of *Cladochytrium tenue.* FIGS. 59 and 60, the rhizomycelium with numerous swellings and incipient zoösporangia. FIGS. 61 and 62, young and empty zoösporangia. FIG. 63, the rhizomycelium with four 2-celled turbinate organs, after Nowakowski (1877).

Karling (1931). In view of the fact that they begin as swellings on the rhizomycelium which may or may not divide, are from one- to many-celled, and exhibit great diversity in size and shape, I shall retain the latter name in this paper. A turbinate organ may thus be composed of one or many so-called collecting cells.

Whether or not the enlargements on the rhizomycelium which Zopf described and figured for *A. rhizidioides* are turbinate organs is obviously open to question and will depend on one's definition of such structures. Because of the great diversity in form, size, and septation exhibited by turbinate organs in general I am inclined to regard them as such. It is not at all certain from Zopf's drawings, however, that food accumulates in them, and that they serve as "Sammelzellen" in the sense of Büsgen.

In *Cladochytrium tenue* (text figs. 58 to 63) according to Nowakowski

(1878) the thallus is essentially similar with numerous fine rhizoid-like branches, swellings, terminal and intercalary zoösporangia. The turbinate organs are more definite than in the preceding genus, and as is shown in text figure 63, frequently occur in succession on the rhizomycelium. Text figures 58, 59, and 60 show their origin as spindle-shaped swellings which doubtless later undergo division. At maturity they are often filled with numerous granules and round bodies (text fig. 63). As in *A. rhizidioides* one of the cells frequently enlarges (text figs. 61 and 62) and becomes the zoösporangium. To the genus *Cladochytrium* doubtless belong Zopf's *Cl. polystomum* and another species which I have described (1931) as *Cl. replicatum* from cells of *Eriocaulon septangulare*, although as noted before Fitzpatrick limits the genus to *Cl. tenue*. The thalli of *Cl. polystomum* and



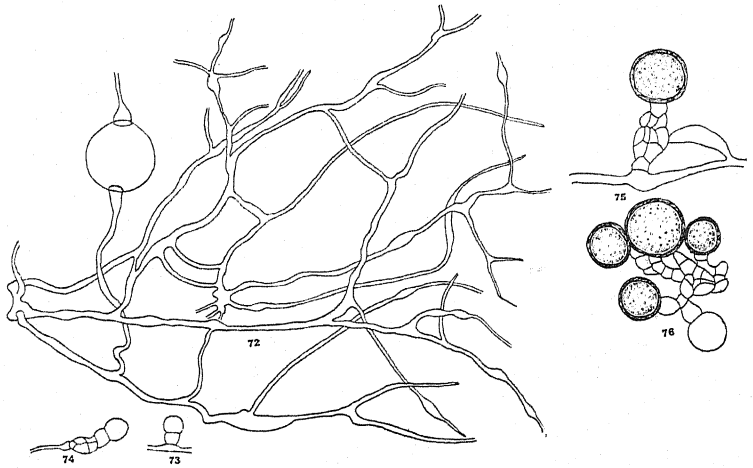
TEXT FIGS. 64–71. *Nowakowskiella elegans*. FIG. 64, the rhizomycelium of *Nowakowskiella elegans* with numerous swellings and incipient zoösporangia. FIGS. 65 and 66, zoöspores and a germinating spore. FIGS. 67–69, stages in the development of intercalary and terminal zoösporangia. FIGS. 70 and 71, irregular and empty zoösporangia, after Matthews (1928).

Cl. replicatum are shown in text figures 82 to 89. The former species is imperfectly known, but Zopf's drawings show a rhizomycelium and intercalary and terminal zoösporangia. Similarly, the thallus of *Cladochytrium replicatum* (text figs. 83–89) consists of a well defined, delicate, more or less hyaline, branched rhizomycelium of variable diameter, extending from one host cell to another, turbinate organs of various size, and terminal and intercalary zoösporangia. It is often quite profusely distributed in the host tissue, but it is difficult to determine whether its profuseness is the result of a single or several zoöspore infections. Because of the opacity of the host cell and the optical homogeneity of the thallus, it is impossible to trace a single infection for a great distance.

The turbinate organs in this species are very diverse in shape, size, and septation (text figs. 84–89). They may be uni- or multicellular, symmetrical

or asymmetrical, large or small, isolated in succession on the rhizomycelium or occur in conjunction with the zoösporangium. In young stages the cells are filled with a more or less hyaline, vacuolated cytoplasm, but when mature this is replaced by a coarser more granular and globular material. Some of the cells appear quite empty, while others are filled with bodies of various sorts.

In the genus *Nowakowskiella* the rhizomycelium becomes very profuse, coarse, and mycelium-like, according to Nowakowski (1877), Butler (1907), and Matthews (1928). Butler describes and figures the filaments as being thick-walled, hyaline, branched, and frequently anastomosing. This genus should perhaps be deferred to later discussion as having the climax type of thallus, but I am taking it up at this point because the type species, *N. elegans*, was first included by Nowakowski in *Cladochytrium*. The thallus

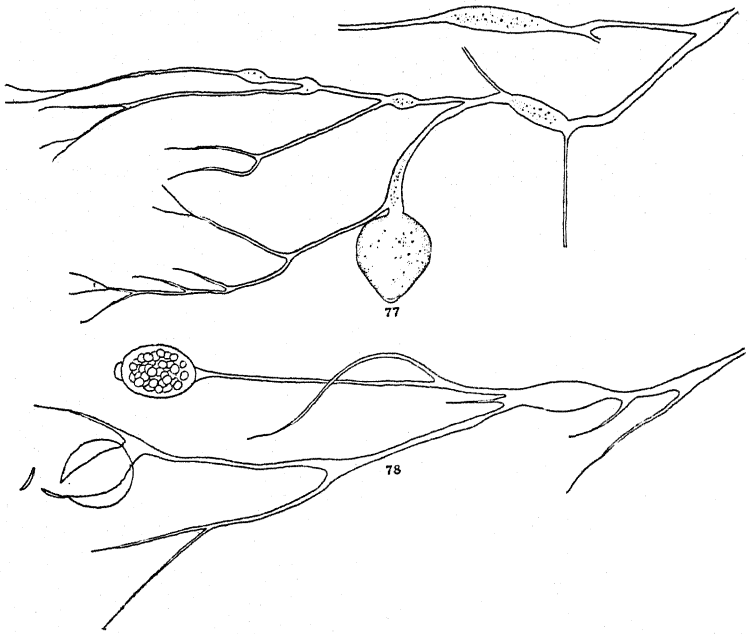


TEXT FIGS. 72–76. *Nowakowskiella ramosa*. FIG. 72, anastomosing rhizomycelium with enlargements and incipient zoösporangia. FIGS. 73–76, stages in the development of the resting spores, after E. J. Butler (1907).

of *N. elegans* as figured by Nowakowski is shown in text figures 77 and 78. The rhizomycelium appears fairly coarse, but runs out to fine points at the extremities. It has numerous swellings or enlargements, so that it is quite irregular in diameter, particularly in the regions of the branches where large junctions are made. The same species was found in 1928 by Miss Matthews in America growing on boiled grass stems (text figs. 64–71). As she figures it, the rhizomycelium spreads profusely from the point of infection and develops numerous intercalary and terminal zoösporangia (text figs. 64, 67, 68), which begin as slight enlargements. In the case of the terminal zoösporangia the portion of the rhizomycelium immediately adjoining swells up as an apophysis, while in the intercalary ones it enlarges on both sides so that the mature sporangium (text figs. 69 and 71) resembles somewhat the

zygospore of *Rhizopus* with its enlarged suspensor cells. Anastomosis of the filaments (text fig. 64) also occurs.

Three additional species, *N. endogena*, *N. ramosa*, and *N. obscura*, have been added to the genus by Constantineanu (1901), E. J. Butler (1907), and Sparrow (1931). The thallus of the second species is shown in text figures 72 to 76. It is quite coarse, irregular in diameter, richly branched, anastomosing and essentially similar to that of *N. elegans*. The terminal and intercalary zoösporangia arise in the same fashion. Butler has also shown the development of the resting spores (text figs. 73–76), from marginal or terminal cells of proliferating "hyphae."

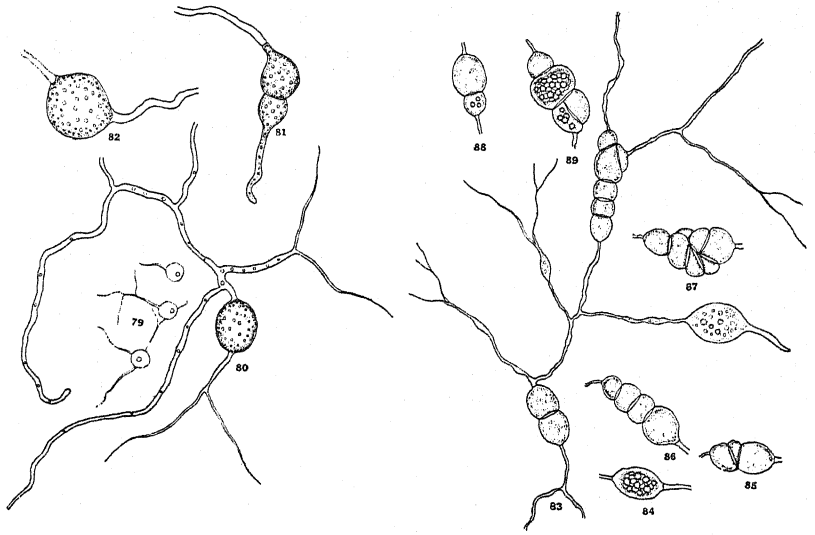


TEXT FIGS. 77 and 78. Rhizomycelium with swellings and proliferating zoösporangia of *Nowakowskiella elegans*, after Nowakowski (1877).

As to the presence of turbinate organs in this genus some doubt has already been expressed (Karling, 1931). That enlargements or swellings of the rhizomycelium which are in no way connected with sporangium formation occur is clearly indicated in the various drawings of Nowakowski, Butler, and Miss Matthews. They may or may not be delimited from the remainder of the thallus by septa. From the drawings presented they appear less definite as cells or organs than in *Cl. tenue*. Butler interprets the profuse proliferation of the "hyphae" in relation to resting spore formation as an extreme development of the "Sammelzellen." In this process the contents of the initial cells, according to Butler, go into the formation of new ones, which is very suggestive of the manner of secondary and tertiary

turbinate cell formation in *Urophlyctis*. I have since examined the thallus of the species of *Nowakowskiella* described by Sparrow which he kindly sent me for inspection, and I am convinced from this material that turbinate organs are present in this genus. The majority of enlargements are continuous with the remainder of the rhizomycelium and perhaps less definite, but clearly delimited, septate organs are also present.

We may next turn to a consideration of the thallus of *Physoderma*. The limits of this genus are poorly defined. Magnus (1901), Bartlett (1926), Gäumann (1926) and Gäumann and Dodge (1928) characterize it by the possession of spherical or slightly ellipsoidal resting spores, while von Minden



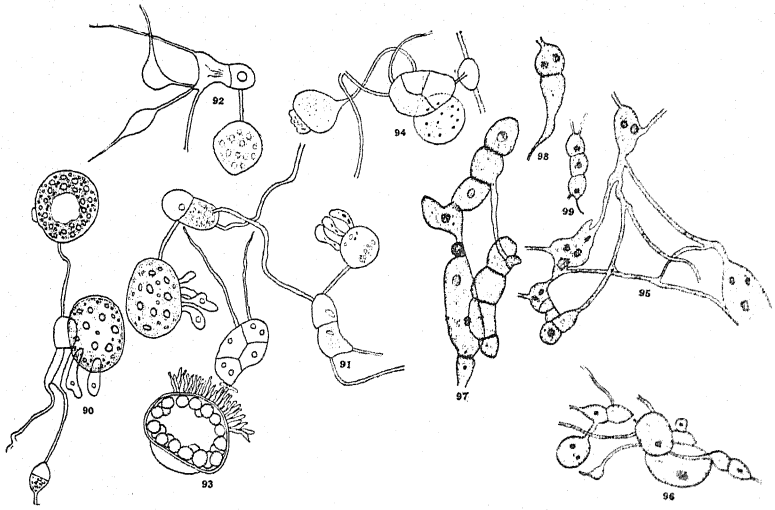
TEXT FIGS. 79–89. *Cladochytrium polystomum* and *Cladochytrium replicatum*. FIGS. 79 and 80, germinating zoöspores and rhizomycelium. FIGS. 81 and 82, developing zoösporangia of *Cl. polystomum*, after Zopf (1885). FIG. 83, the rhizomycelium with turbinate organs and incipient zoösporangia of *Cladochytrium replicatum*. FIGS. 84–89, variations in the size, shape, septation, and content of the turbinate organs, after Karling (1931).

and Fitzpatrick limit it to those species which cause only slight discoloration or malformation of the host tissue. As illustrative examples I have chosen *Ph. Butomi*, *Ph. maculare*, and *Ph. Zeae Maydis* as they have been figured by Büsgen (1887), Clinton (1902), and Tisdale (1919). In all of these species (text figs. 90–99) the more filamentous portion of the thallus is very delicate and evanescent, so that in dried specimens very little but turbinate organs and resting spores are to be found.

In species of this genus whose complete life history is known two types of development and thallus organization are present. This is particularly evident of *Ph. maculare* in which ephemeral or temporary zoösporangia have been found in addition to resting spores.

The formation and development of the ephemeral zoösporangium in

this species as described by Clinton is quite different from that of the turbinate organs, resting spores, and the remainder of the rhizomycelium. The zoöspore comes to rest on the surface of the *Alisma* leaf and forms a small penetrating tube which enters the host cell. There it develops into a rhizoidal system, while the zoöspore itself grows, enlarges, and finally becomes a large folded or lobed zoösporangium. Thus at maturity the entire structure looks like the thallus of a partially extramatrical species of the Rhizidiaceae with an internal rhizoidal system and a superficial zoösporangium. According to Clinton's description and figures it is in no way connected with the more filamentous rhizomycelium. Like the thallus of the



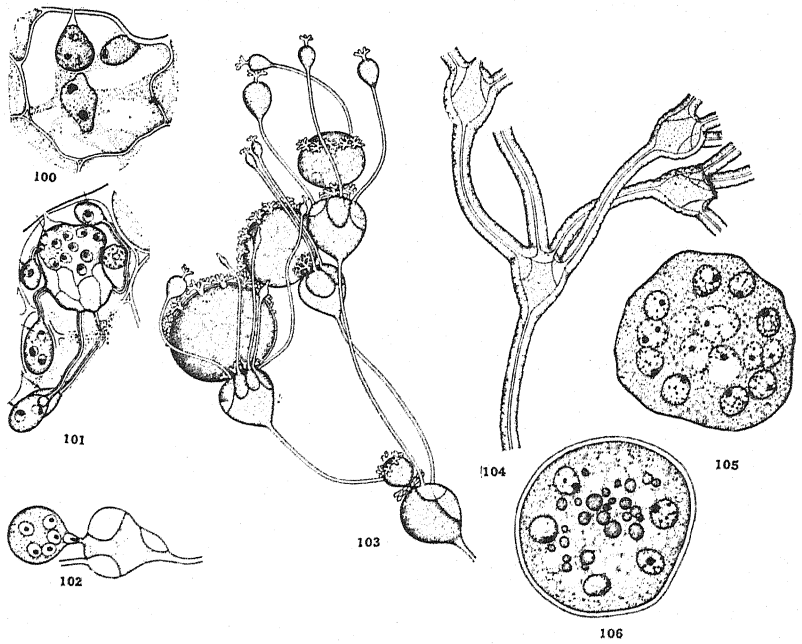
TEXT FIGS. 90–99. *Physoderma Butomi, Ph. maculare,* and *Ph. Zeae Maydis.* FIGS. 90 and 91, rhizomycelium with turbinate organs, haustoria, and incipient resting spores of *Ph. Butomi.* FIGS. 92 and 93, young and mature resting spores, after Büsgen (1887). FIG. 94, a germinated zoöspore of *Ph. maculare* which has formed a primary and secondary turbinate organ, and a young resting spore, after Clinton (1902). FIGS. 95 and 96, the irregular rhizomycelium of *Ph. Zeae Maydis* with turbinate organs and young resting spores. FIGS. 97–99, variations in the size, shape, and septation of the turbinate organs, after Tisdale (1919).

former family it is accordingly monogenocentric in development and organization. If Clinton's observations are correct the presence of a monogenocentric stage in the life history of *Ph. maculare* suggests perhaps a close relationship to the Rhizidiaceae.

The successive developmental stages of the rhizomycelium, on the other hand, are quite similar to those described for *Cladochytrium* and have been well figured in a number of species. The germinating zoöspore forms almost at once a swelling or turbinate organ (Büsgen, figs. 5, 6, 7, and 9, Pl. 15;

Clinton figs. 25–31, Pl. 3; and Tisdale, Pls. 14 and 15) at the end of the penetration tube, which becomes a primary center for further development. The zoöspore case and penetration tube in the meantime degenerate and disappear. From the primary center or turbinate organ grow out filaments, resting spores, and other turbinate organs (text fig. 94) which then become secondary centers. By homoeotic development the thallus or rhizomycelium is thus reduplicated in structure and appearance as it spreads from one host cell to another or within the same cell, and becomes thereby a polycentric system.

The turbinate organs in this genus are most variable in size, shape, and septation, as is shown in these text figures. They may be very irregular in

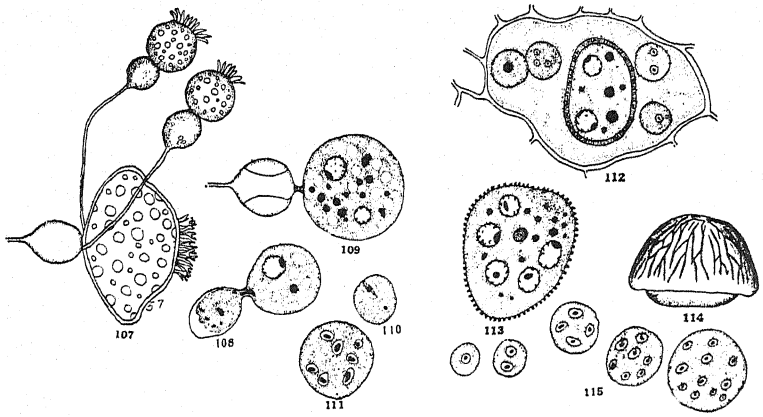


TEXT FIGS. 100–106. *Urophlyctis Alfalfae*. FIG. 100, a primary multinucleated turbinate organ. FIG. 101, a primary multicellular turbinate organ from which have been developed five secondary turbinate organs. FIG. 102, a stage showing the origin of the resting spore from a turbinate organ. FIG. 103, the thickened rhizomycelium and turbinate organs. FIG. 104, a portion of the thallus. FIGS. 105 and 106, sections of resting spores, after Jones and Dreschler (1920).

shape and from 1- to 4-celled (text figs. 91, 92, and 95). Figures 98 and 99 show turbinate organs which are strikingly similar to the conidia of phragmosporous Fungi Imperfecti. The bluntly lobed and coarsely branched structures at the apex of the turbinate organs and resting spores of this and the following genus are undoubtedly haustoria. They are anatomically identical with the haustoria of many of the higher fungi described above; but their relation to the host protoplasm is practically unknown, since these

parasites have received very little attention from the cytological viewpoint of parasitism. As contrasted with the turbinate organs and resting spores the formation of these haustoria is distinctly heterotic.

The same type of thallus structure and organization is characteristic of the genus *Urophlyctis* also. In fact the thalli of the two genera are so similar in most respects that very few authors agree as to which genus a species belongs. *Urophlyctis Alfalfae* and *U. Potteri*, however, show considerable minor structural differentiation from the *Physoderma* species described above; and since they have been studied cytologically by Jones and Dreschler (1920) and Bartlett, I shall use them as illustrations of the genus (text



TEXT FIGS. 107–115. *Urophlyctis Potteri* and *U. Alfalfae*. FIG. 107, a portion of the thallus of *U. Potteri* with a mature resting spore, three turbinate organs, and two incipient resting spores. FIGS. 108 and 109, stages in the formation of the resting spore. FIG. 110, cross section of a turbinate organ showing the division of the nucleus. FIG. 111, cross section of a multinucleated turbinate organ. FIGS. 112–114, sections of resting spores and a mature spore, after Bartlett (1926). FIG. 115, stages in nuclear multiplication of the resting spore of *U. Alfalfae*, after Fron and Lasnier (1920).

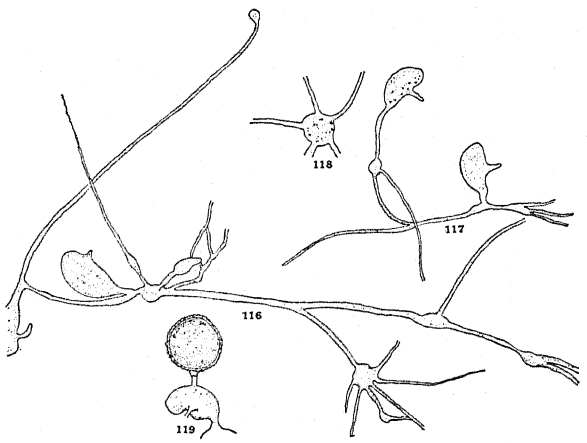
figs. 100–115). Ephemeral or temporary zoösporangia of the type found in *Ph. maculare* have not so far been reported in the literature, and asexual monogenocentric thalli are thus unknown.

In *U. Alfalfae* the zoöspore apparently forms a penetration tube which enlarges at its tip into a flagon-shaped cell as in the previous genus (text fig. 100). Its nucleus undergoes division several times, as in figure 110 of *U. Potteri*, and the cell thus becomes multinucleated (text figs. 100 and 101). Tangential segments or discs are then cut off from the mother cell, so that the turbinate organ becomes multicellular. From most of these segments grow out filaments which form secondary turbinate organs at their tips, and these in turn form tertiary and quaternary ones, as is shown in text figure 103. In this manner by homoeotic development the system is replicated almost *ad infinitum*. The central or sporogenous cell of each turbinate gives rise to a bud which develops into the resting spore (text figs.

102, 108, and 109). The turbinate organs are thus the vegetative centers for the formation of successive resting spores, haustoria, filaments, and additional turbinate organs.

The filamentous portion of the rhizomycelium of the Cladochytriaceae has been described as rather delicate and evanescent in most species, but in *U. Alfalfae* (text fig. 104) it may become extremely coarse and thickened with maturity. This is a further indication that fineness or delicate structure is not the outstanding characteristic of the rhizomycelium as well as rhizoids.

The thalli of three more genera remain to be considered. *Rhizidiocystis*, *Hyphochytrium*, and *Catenaria* have been considered as doubtful chytrids by many students, since they show considerable divergence in structure and are also imperfectly known. In text figures 116 to 119 I have attempted to reproduce from photographs portions of the thallus of *Rhizidiocystis*. According to Sideris (1928) the rhizomycelium is very abundant in the form of delicate filaments with numerous enlargements, kidney-shaped zoösporangia, and round resting spores. The swellings or enlargements are very indefinite in shape as in *N. ramosa*, but as is shown in text figures 116 and 118, they doubtless serve as vegetative centers and are thus comparable to the turbinate organs of the previous genera. However, Sideris's photographs of the thallus as well as his descriptions of sporangium germination



TEXT FIGS. 116–119. *Rhizidiocystis Ananasi.* FIGS. 116 and 117, the rhizomycelium of *Rhizidiocystis Ananasi* with incipient kidney-shaped zoösporangia and turbinate organs. FIG. 118, a single turbinate organ. FIG. 119, formation of the resting spore, after Sideris (1929).

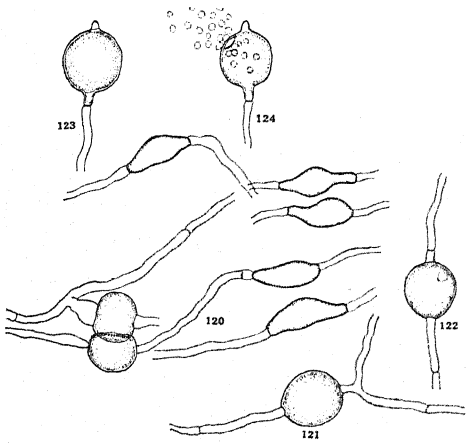
and resting spore formation are not sufficiently clear to warrant a wide generalization.

The thallus of *Hyphochytrium* as figured by Zopf (text figs. 120–124) is very coarse and septate and mycelium-like with numerous intercalary and terminal zoösporangia and swellings (text fig. 120). The latter may perhaps be one-celled turbinate organs. As to whether or not *Hyphochytrium* be-

longs in the Cladochytriceae or the chytrids at all is doubtless open to question, but the organization of its thallus is very similar in many ways to those of the other genera described above.

In superficial appearance the thallus of *Catenaria* is not very similar to those of the previous genera, but its ontogenetic development is closely parallel, according to Butler and Buckley (1928). In text figures 125 to 138 I have shown the thallus of *C. Anguillulae* as it has been figured by Villot (1874), Dangeard (1885), E. J. Butler (1928), Butler and Buckley (1928), and Buckley and Clapham (1929). It consists of a dichotomously branched rhizomycelium composed of oval and irregular zoösporangia (text figs. 127 and 128) and numerous finely branched ends. The zoösporangia are set off from the remainder of the rhizomycelium by septa and separated from each other by narrow isthmuses, so that they occur in more or less close succession in the thallus. When growing in infusorial cysts and rotifer eggs the thallus is so compactly crowded, however, that it is almost impossible to distinguish its different elements.

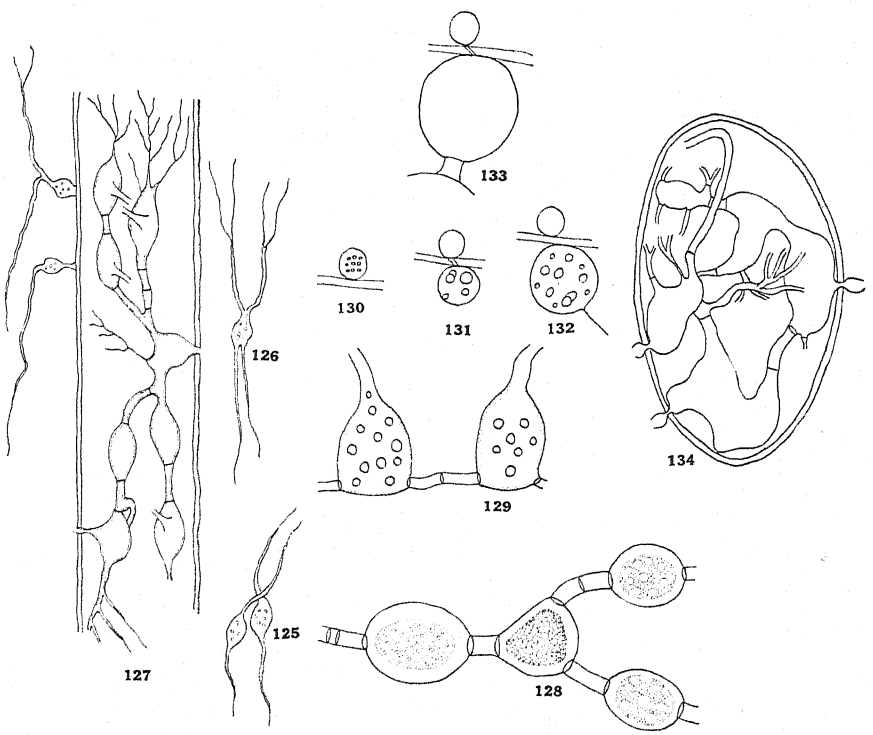
In cells of *Nitella* Dangeard describes and figures germinating zoöspores (text figs. 125 and 126) with several germ tubes, but in the infection of liver fluke ova Butler and Buckley have shown (text figs. 130–133), that as soon



TEXT FIGS. 120-124. *Hyphochytrium infestans*. FIG. 120, a portion of the septate thallus with several incipient zoösporangia. FIGS. 121–124, mature intercalary and terminal zoösporangia, after Zopf (1885).

as the penetration tube enters the host cell it enlarges at its tip and becomes the primary incipient zoösporangium. From this cell are then developed rhizoids and secondary zoösporangia. In this respect the development of the thallus is very similar to *Physoderma* and *Urophlyctis*. E. J. Butler (1907), on the other hand, describes (fig. 13) the rhizomycelium as being rather filamentous at first throughout the ovum and then enlarging at various intervals (fig. 14) to form zoösporangia. It is to be noted, however,

that neither he nor Dangeard figures or describes the initial infection stages. It is thus evident that further studies are needed to clarify the successive developmental stages of the thallus. Liver fluke ova and infusorial cysts are difficult host material for the study of this problem, since the thallus becomes so crowded that its various elements can scarcely be differentiated. Since the organisms spread readily to *Nitella* it is perhaps only in such host cells that the stages can be correctly observed. As to the presence of defin-

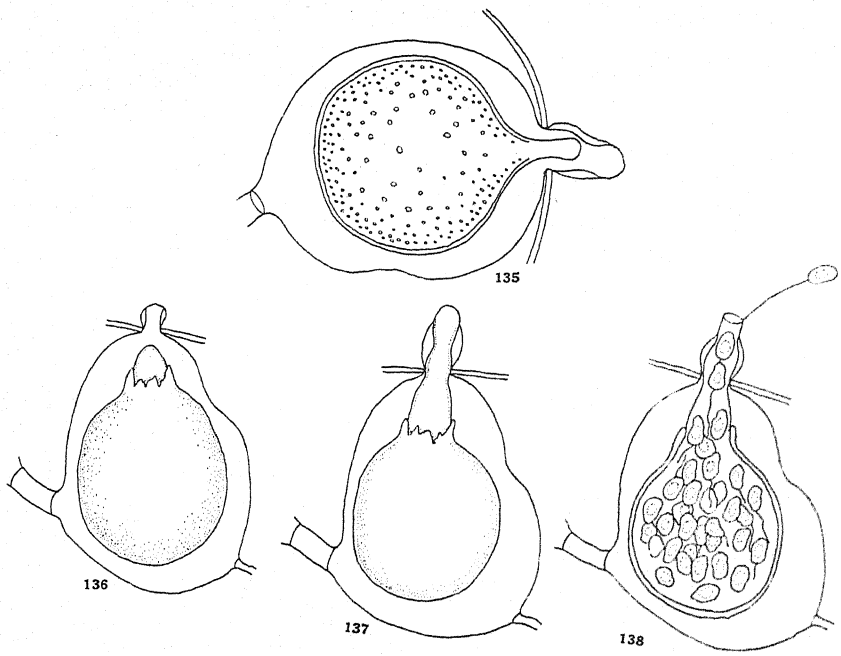


TEXT FIGS. 125–134. *Catenaria Anguillulae.* FIGS. 125–127, germinating zoösporesand mature thallus with zoösporangia, after Dangeard (1885). FIGS. 128–129, mature thalli, after Villot (1874). FIGS. 130–133, stages in the development of the thallus in liver fluke ova after Butler and Buckley (1927). FIG. 134, the mature thallus in a liver fluke ovum, after E. J. Butler (1928).

ite turbinate organs in *Catenaria* the evidence from the literature is not very conclusive. Irregularities and swellings on the rhizomycelium apart from the zoösporangia have been figured, but that they are the centers of thallus replication as in *U. Alfalfae*, for instance, remains in my opinion to be demonstrated from future studies. Butler and Buckley have nevertheless shown that they are closely associated with the formation of zoösporangia and rhizoid-like branches, and in this relation they may perhaps be regarded as centers. The exact morphological significance and function of turbinate organs in general thus remains somewhat obscure.

This concludes the survey of the chytrids which possess a rhizomycelium. Doubtless other less certain genera and species might be included, but sufficient forms have been considered to emphasize definitely the view which was expressed in the beginning; namely, that as the thallus spreads from one host cell to another or in the substrate on which it is growing it is replicated by the establishment of new and successive centers of growth and differentiation. The successive centers are almost exact replicas of the primary ones, and homoeosis thus reaches full expression.

The thallus of the Cladochytriaceae is nevertheless as a whole definitely



TEXT FIGS. 135–138. *Catenaria Anguillulae.* FIG. 135, retraction of the zoösporangium to form a resting spore. FIGS. 136–138, stages in the germination of the resting spore, after Buckley and Clapham (1929).

organized. The center of organization is at first the vegetative rhizomycelium as a unit, and for this reason we may speak of the thallus as being *vegetato-* or *trophocentric.* With the coming of maturity, however, and the development of new centers in the form of sporangia and resting spores it becomes reproducto- or genocentric. Moreover, since many reproductive centers are formed the thallus is at maturity a *polygenocentric* system in contrast to the *monogenocentric* thallus of the Rhizidiaceae.

A trophocentric thallus thus becomes genocentric with maturity. This is perhaps better exemplified by the thallus of the higher fungi. The center of organization is the mycelial system as a whole during its feeding, growing, and vegetative stage. Then as it becomes mature many reproductive

organs or centers are formed towards which the accumulated food substances doubtless move, and growth and differentiation for those particular regions are largely localized in developing centers.

Summary

1. The rhizoids of the family Rhizidiaceae are in general distinguishable from the mycelium of higher fungi by their decreasing in diameter from the point of origin and tapering to extreme fineness *without any tendency to form new centers for the processes of growth, differentiation, and reproduction.*

2. In the Myxochytridineae and Rhizidiaceae the zoöspore gives rise to a monocentric thallus. The center of gravity of the thallus is almost at once or shortly transformed into a reproductive structure, and hence the organization of the thallus at maturity may be characterized as being *monoreproducto-* or *monogenocentric.* In cases where the reproductive structure forms non-sexual zoöspores and resting spores the thallus may be distinguished as asexually monogenocentric, while if gametes are produced it is sexually monogenocentric.

3. The thallus of the family Cladochytriaceae is designated as a rhizomycelium to distinguish it from the mycelium of the higher fungi and the rhizoidal system of the Rhizidiaceae. By homoeosis the thallus is replicated as it spreads in the host tissue or in the substrate on which it is growing by the establishment of successive centers of growth and differentiation.

4. In *Cladochytrium* to a certain extent, and in *Physoderma* and *Urophlyctis*, the turbinate organs appear to be the centers of reduplication, since from them are formed the successive resting spores, secondary and tertiary turbinate organs, haustoria, and filaments as the thallus spreads in the host tissue. In other genera the turbinate organs appear to be perhaps less specialized and definite and consequently their significance less obvious.

5. During the feeding, growing, and vegetative stage the thallus of the Cladochytriaceae may be characterized as vegetato- or trophocentric in organization since the center of the system is in the rhizomycelium as a whole. At maturity, however, numerous centers of growth and differentiation in the form of sporangia and resting spores develop and the thallus becomes a polygenocentric system. This type and change of organization with maturity is also characteristic of the thallus or mycelium of the higher fungi.

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INVESTIGATIONS ON THE MORPHOLOGY AND COMPARATIVE HISTORY OF DEVELOPMENT OF FOLIAR ORGANS III. CATAPHYLL AND FOLIAGE-LEAF ONTOGENY IN THE BLACK HICKORY (*CARYA BUCKLEYI* VAR. *ARKANSANA*) [1]

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INTRODUCTION

In the previous article in this series (Foster, 1931 *b*), it was concluded that the fundamental architecture of the cataphyll in the black hickory is that of a simple foliar organ possessing a more or less distinct axial region flanked by two lateral, often asymmetrical wings. This conclusion was based largely, however, upon evidence derived from a study of the comparative form and venation of the adult cataphyll and, in view of my rejection of Cook's (1916, 1923) phylogenetic interpretation of hickory bud scales, shed no light upon the real basis for the morphological divergence between scale and foliage leaf. With the hope that ontogenetic facts might contribute to the solution of this problem, the critical morphogenetic phases in development of the cataphylls and foliage leaf have been studied in detail and the results are presented in the present paper. In view of Domin's (1931, p. 237) insistence that "a morphological problem must be solved primarily by comparative morphological investigations" it is important to note that at least in this specific case, the facts revealed by ontogeny support the conclusions reached upon comparative grounds. It would seem therefore that an undue emphasis of either the comparative-morphological or the ontogenetic methods of attack on a problem of foliar organization is unjustified and that, on the contrary, as Arber (1925, p. 4) points out, all possible lines of evidence must be carefully weighed before a scientific conclusion can be reached.

MATERIALS AND METHODS

As I have explained previously (Foster, 1931 *b*), the terminal buds of spur shoots of the black hickory tend to be less variable as regards scale and foliage leaf content than the terminal buds of long shoots and hence have been almost exclusively used in the present developmental study. In an effort to obtain uniform results, especially as regards the foliar cycle, most of the material used in this investigation was obtained from a single

[1] Contribution from the Botanical Laboratory of the University of Oklahoma NS No. 12.

vigorous young tree growing in oak-hickory woods about six and one-half miles east of Norman, Oklahoma; additional material was secured from trees growing in the vicinity of Tecumseh, Oklahoma. Collections were made at frequent intervals, usually every 3–5 days, beginning with the dormant period in winter and extending through the periods of scale and foliage-leaf formation. The material was preserved in 5 percent formalin, which toughens it sufficiently for careful dissection.

The dissection was done with the aid of a Spencer binocular microscope at a magnification of 61.2 diameters. During dissection, it was found necessary to keep the material thoroughly moistened with water which was applied with a medicine dropper. Measurements of scale and foliage-leaf primordia were made with the aid of an ocular micrometer used in connection with a calibrated microscope.

For purposes of emphasis and clarity, no attempt has been made in the figures illustrating this paper to depict the relative positions of *all* the developing foliar organs of the bud at each ontogenetic stage shown. On the contrary, the illustrations in most instances show only the critical developmental stages of the *youngest* foliar organs in the vicinity of the growing point. For a general scheme of bud construction, the reader is referred to text figure 1 in the previous paper in this series.

Periodicity of Foliar Development on the Spur Shoot

In the preceding paper (Foster, 1931 *b*) it was pointed out that the "foliar cycle" on spur shoots consists usually of two upper transitional forms, seven bud scales, and four foliage leaves. Deviations from this average condition do occur but in general this cycle of development is repeated annually and is only interrupted normally by the appearance of the terminal female inflorescence. Before the comparative ontogeny of the foliar organs produced during the annual cycle can be clearly appreciated it will be essential to show, as accurately as possible, the periods during which cataphylls and foliage leaves are laid down by the growing point.

In an attempt to secure uniform results, material was collected mainly from one tree which appeared to be normal in vigor and habitat. Different results, as regards the exact time of organ formation, might have been obtained from other trees, or from the same tree during a different year; these facts should be borne in mind in evaluating the data presented in table 1, which admittedly are relative and not absolute in character.

If one dissects a terminal leaf bud during the rest period (November to March), the primordia of the upper transitional forms are usually evident at the growing point. This recalls the situation obtaining in the winter bud of *Aesculus Hippocastanum* (Foster, 1929) and emphasizes the definiteness of the foliar cycle in the black hickory. As is shown in table 1, the

TABLE I. *A Comparison of the Periods of Initiation and Morphogenetic Development of the Foliar Organs of a Terminal Bud with the General Processes of Growth in the Main Shoot*

Date	Old Terminal Bud (1929)	New Terminal Bud (1930)
Feb. 19	Apparently dormant.	Primordia of upper transitional forms present at growing point.
March 22	Inner bud scales becoming accrescent.	Primordia of upper transitional forms rapidly growing. Primordium of 1st (and sometimes 2d) scale appearing.
April 4	Inner bud scales rapidly enlarging; foliage leaves still enclosed within scales.	Primordium of 1st scale growing rapidly; primordium of 2d scale papillate.
April 9	Tips of foliage leaves emerging from "tube" formed by accrescent scales.	1st scale developing wings; primordium of 2d scale enlarging; primordium of 3d scale appearing.
April 12	Upright cluster of unexpanded foliage leaves exposed.	2d scale developing wings; 3d scale with marginal growth initiated; primordium of 4th scale appearing.
April 15	New shoot elongating. Foliage leaves expanding; bud scales reflexed; new terminal bud visible.	3d and 4th scales developing wings; primordium of 5th scale initiating marginal growth; primordium of 6th scale papillate.
April 18	New shoot well advanced; upper cataphylls still persistent.	5th scale developing wings; primordium of 6th scale initiating marginal growth; primordium of 7th scale papillate.
April 21	Upper cataphylls still persistent.	6th scale developing wings; primordium of 7th scale initiating marginal growth; primordium of first foliage leaf papillate.
April 23	Upper cataphylls still persistent.	7th scale developing wings; primordium of first foliage leaf elongating but unsegmented. Primordium of second foliage leaf papillate.
April 26	Cataphylls beginning to fall; upper one or two only persistent.	Little change except suggestion of leaflet initiation in primordium of first foliage leaf.
April 30	Cataphylls gone.	1st foliage leaf with 2(3) prs. lateral leaflet primordia; primordium of 2d foliage leaf elongating; primordium of 3d foliage leaf papillate.
May 3		1st foliage leaf with elongating leaflet primordia; lateral leaflet primordia appearing in 2d foliage leaf; primordium of 3d foliage leaf elongating but unsegmented.
May 8		Little change save rapid increase in size of leaflet primordia of 1st and 2d foliage leaves and blade initiation on upper leaflets of 1st.
May 13		1st, 2d and 3d foliage leaves rapidly growing; primordium of 4th foliage leaf elongating; papillate primordium of 1st upper transitional form just appearing.

period of bud swelling (March 22) is accompanied by the rapid growth of the upper transitional forms and the initiation, at the growing point, of the primordium of the first bud scale. We may now consider briefly the period of scale formation in the new terminal bud.

Reference to table 1 shows that the period of scale formation extends from March 22 to April 18, thus covering a time interval of 28 days. It is instructive to compare on similar dates the behavior of the expanding shoot with the stage of development of the new terminal bud at its apex. For example, from March 22 to April 12, a period of 22 days, scale initiation is proceeding rapidly at the growing point although the new terminal bud is still protected by the cluster of unexpanded foliage leaves. During this period, the cataphylls of the expanding bud (formed the previous year) have become accrescent and reflexed and the process of elongation in the shoot has begun. On April 15, however, the foliage leaves are rapidly expanding and reflexing and the new terminal bud, consisting of six rapidly-growing scales, is now exposed directly to the atmosphere. The primordium of the seventh scale appears at the growing point about April 18 and the period of scale formation in the new terminal bud is usually complete. It may prove significant, in future developmental-physiological studies, to bear in mind that at least in the black hickory, the completion of scale formation in the new terminal bud appears to coincide with the general cessation in growth of the main shoot on which it is borne.

Table 1 indicates that the primordium of the first foliage leaf appears at the growing point on April 21 and that the general period of foliage-leaf initiation extends from this date to May 13, a time interval of 22 days. During this period, the reflexed cataphylls at the base of the shoot gradually wither and are finally deciduous. On May 13, the new terminal bud was found to consist of two upper transitional forms, seven bud scales, four foliage leaves (in successive stages of development) and, at the growing point, the papillate primordium of the first upper transitional form of the bud destined to develop during the spring of 1931.

A good deal of variation, as regards organ formation, obtains after May 13, in the material investigated. Perhaps most frequently the primordium of the second upper transitional form appears and then the growing point becomes inactive until the following spring. Variations in the number of foliage leaves formed in the various buds, however, on a single tree make it impossible to define accurately the exact period during which the primordia of the upper transitional forms are laid down. It seems clear, however, that by the middle of May, organ formation has ceased at the growing point, and further development in the new terminal bud consists in the enlargement and histological specialization of the cataphylls and foliage leaves.

A final point of importance is suggested by a careful analysis of the data presented in table 1, *viz:* that the scale primordia are laid down at the growing point at a relatively faster rate than are the foliage-leaf primordia.

During the period of scale formation, which lasts about 28 days, seven primordia are formed, or in other words, the plastochron is four days in length. The period of foliage leaf formation, in contrast, lasts about twenty-two days during which time four primordia are formed, thus giving a plastochron of five and one-half days. The meaning of this apparent difference in the rate of scale and foliage leaf initiation, however, is far from evident at present. According to Schüepp's (1929, pp. 792–793) analysis of the data on foliar periodicity appearing in contributions from Blaauw's laboratory, the scales are formed at a faster tempo than the foliage leaves, suggesting rapid growth. In *Acer Pseudoplatanus*, however, the primordia of scales and leaves appear at about the same tempo. Evidently considerable variation, according to the plant, is to be expected. Schüepp points out in discussing this variation, that the true measure of the activity of the growing point is the velocity of multiplication of the promeristem cells and not the length of the plastochron. The latter, according to Schüepp, is not only dependent upon the velocity of growth but also upon the size-relationship between the youngest foliar primordium and the constantly "regenerating" portion of the growing point. Perhaps further study of the phenomenon from this standpoint may lead to significant discoveries.

Cataphyll and Foliage-leaf Ontogeny

In view of the detailed account which has already been given (Foster, 1931 *b*) of the adult form and organization of the cataphyllary structures and the foliage leaves in the black hickory, it will be necessary at this point to review briefly only the salient features of these organs. It will be recalled that with the exception of the lower transitional forms, the outer of which usually possesses a rudimentary laminar primordium, the cataphylls are organs with a relatively simple morphological organization and exhibit a surprising range in form, which varies from ovate-acute or obovate-emarginate in the lower scales to spatulate, linear, or linear-rhomboid in the inner scales. In contrast to the cataphylls, the foliage leaf is morphologically complex, and consists of a pulvinus-like leaf base, a petiole, and an imparipinnate lamina of 3–7 leaflets. The facts presented in the previous paper, however, shed no light upon the dynamic aspects of this striking divergence in form and organization between the adult scale and foliage leaf, because the critical morphogenetic phases of development of these organs were not considered. The changes in size and form of the foliar organs during bud expansion are important but are secondary in importance as compared with the early phases of development occurring in the vicinity of the growing point, when the organ primordia are but a fourth to a third of a millimeter in height.

In the present section of this paper the critical stages in the ontogeny of the successive foliar organs of the terminal bud will be described in the

order of their development, beginning with the configuration of the primordia of the lower transitional forms as they appear at the growing point of the winter bud and ending with the initiation of the last foliage-leaf primordium. In this account, an attempt will be made not only to compare graphically the significant morphogenetic developmental stages of the cataphylls and foliage leaves but also to emphasize that the appearance of these organs in the life cycle is intimately related to the rhythmical growth and general organization of the shoot.

The Upper Transitional Forms

In correlation with the variation in the morphological organization of the upper transitional forms, which has already been described (Foster, 1931 *b*), it is impossible to generalize regarding the form of the primordia of these organs in the winter bud. Figure 1, Plate II, shows a not infrequent condition in which the larger of the two primordia shows no evidence of lateral leaflet primordia; it seems probable that such an initial would develop into the ovate-acuminate type which I have already described (Foster, 1931 *b*, Pl. LXI, fig. 2). Perhaps more frequently, however, the primordium of the outer of the two upper transitional forms consists of a short, thick basal region and three tiny leaflet primordia (Pl. II, fig. 2). Regardless of the presence or absence of lateral leaflet primordia in the initial of the outer transitional form, the primordium of the second transitional form, in all cases that I have examined, appears at the growing point of the winter bud as a thick, broad-based, dorsally-convex, ventrally-plane structure and, in these respects, resembles the young primordia of the bud scales proper (figs. 1 and 2).

Coincident with the swelling of the terminal bud, the primordia of the upper transitional forms develop rapidly and the growing point begins to form the scale primordia of the new terminal bud (table 1). Following the stage shown in figure 2, the basal region of the outer of the two transitional forms grows actively in length and basal width and begins to form massive lateral wings; during this phase in development, the rudimentary laminar primordium slowly increases in size and blade-formation is initiated on the ventral surfaces of each leaflet primordium in the form of two delicate lateral meristematic weals (fig. 3). The further development of the outer transitional form involves the continued increase in the length of the basal region and the extent of its rapidly growing wings; usually the differentiation of the laminar primordium ceases after the stage just described. It is interesting to note here that Langdon (1931, p. 290), in a study of the scales of the primary axis of seedlings of *Carya cordiformis*, finds that during the early stages of development "the laminar portion of the scale primordium parallels that of the foliage leaf. Shortly after the formation of the first three primary segments, dominant growth commences in the sheathing portion

of the appendage, preventing further elaboration of the lamina." It is evident from Langdon's figures (Pl. II, figs. 15 and 16) that these epicotylar scales in *Carya cordiformis* are similar in organization to the outer of the upper transitional forms in the black hickory. Information is needed, however, on the development and structure of the cataphylls of the winter terminal and axillary buds in *Carya cordiformis* before further conclusions of a comparative nature may be drawn. The inner of the two transitional forms, because of its failure to differentiate lateral leaflet primordia, pursues a much simpler development. Following the stage shown in figure 2 the primordium of this organ experiences a rapid increase in basal width and height. When viewed laterally at this developmental stage, the inner transitional form is seen to consist of a rapidly extending apex (which becomes the acuminate pointlet of the mature organ) and a basal region with massive, actively growing lateral wings (fig. 3). A ventral aspect, at the same stage, shows that the prominent wings of the inner transitional form extend from the base nearly to its apex, a fact which clearly excludes the possibility of drawing rigid transverse delimitations of morphological "elements" in such an organ (figs. 4 and 5). The persistence of wing-development, and the slow increase in height and basal width in the inner transitional form leads to the ovate-acuminate form of this organ at maturity.

During the ontogeny of both of the upper transitional forms, the median central region becomes noticeably thickened and distinct from the lateral wings; this localized growth in thickness leads to the prominent and characteristic dorsal keel of the adult organs. Hairs and colleters appear early on the dorsal and apical regions of the upper transitional forms, as in the case of the bud scales proper (fig. 5).

The Bud Scales Proper

Before describing in detail the ontogeny of the successive bud scales, it will be well to emphasize certain early developmental features shared by all the scale primordia. The scale initials appear acropetally at the growing point, apparently with a 2/5 divergence [2] and, at the earliest visible stage, have the form of rounded or hemispherical protuberances. An interesting feature of the spiral arrangement of the scale primordia is the fact that the youngest initial, at a given developmental stage of the bud, does not lie midway between the two next older primordia but is invariably displaced towards the larger one (cf. fig. 5, Pl. II and figs. 15 and 17, Pl. III). This situation is not in accord with "Hofmeister's Rule" (cf. Hofmeister, 1868, pp. 482–483) but, on the contrary, seems closely analogous to the position of the foliar organs of the terminal bud of the seedling of *Helianthus annuus* (likewise with a 2/5 divergence) as describedly by Goebel (1928, pp. 281–282, fig. 288).

[2] Foster, 1931*b*, text figure 1.

Following the early papillate stage of development, the bud scale primordia rapidly increase in basal width, thickness and height, finally reaching what I have chosen to call the "determinate stage" since from this time onward, their ontogeny very definitely proceeds along utterly different lines from those pursued by a foliage-leaf primordium. A highly significant aspect of the early stages in growth of a scale primordium lies in the fact that its basal width increases at a relatively faster rate than does its height. Indeed, if this inequality in growth rates is computed by dividing the height [3] of the primordium by its basal width,[4] a ratio of less than unity is invariably obtained with the exception of the primordia of the seventh scale. As I shall show later, this ratio is the clear evidence of the almost initial divergence in mode of growth of the scale and foliage-leaf primordia.

In view of certain important differences in the early ontogeny of the outer and inner bud scales, it will be necessary to discuss their development separately, although the distinction between "outer" and "inner" scales is admittedly arbitrary.

The Outer Bud Scales (1–3 Inclusive)

The hump-like primordium of the first scale appears at the side of the growing point during the early period of bud swelling (cf. table 1 and fig. 3, Pl. II). As the result of rapid increase in basal width, thickness, and height, the primordium quickly reaches the determinate stage where it appears as a thickish, broad-based, ovate structure, dorsally convex and ventrally only slightly concave (fig. 4). Almost simultaneously with the appearance of rapidly-elongating hairs on the median-dorsal surface, two important growth processes appear in the primordium. In the first place, its apex begins to grow rapidly. Secondly, and of the greatest importance, active and uniform meristematic activity ensues along its ventral margins (figs. 5 and 6). Up to and including the developmental stage just described, the ratio of height to basal width is less than unity, and varied in the seven primordia measured from .5–.8. Following the ontogenetic stage depicted in figures 4, 5, and 6 of Plate II, the first scale elongates rapidly and as the result of active marginal growth develops prominent lateral wings which, growing towards one another, finally overlap above the base and eventually enclose the developing primordia of the second and third scales (figs. 7 and 8). The first scale has now passed through the critical morphogenetic period of development and in form is an ovate-acute, conical structure with rapidly broadening wings and a slowly thickening median central region. As is shown in figures 6, 7, and 8, coarse hairs continue to appear and to develop rapidly on the median-dorsal and apical regions of the first scale; it will be noticed that the rapidly-growing wings are quite glabrous during the early phases of their development.

[3] That is, the length of a median line joining the apex and base on the ventral surface.

[4] That is, the length of a line connecting the basal margins on the ventral surface.

Coincident with the beginning of marginal growth in the primordium of the first scale, the second scale appears at the side of the growing point as a rounded papilla (figs. 5 and 6). As in the case of the first scale, the primordium of the second scale first of all increases rapidly in size, gradually losing its early papillate form, and appearing in the determinate stage as a broad-based thickish initial in which apical and ventral-marginal growth are proceeding actively (figs. 7 and 9). During the early phases of its growth, the basal width of the primordium of the second scale increases more rapidly than its height, the ratios computed from four different initials measured varying from .5–.75. The more or less uniform and rapid growth at the ventral margins of the primordium, after the determinate stage has been reached, coupled with an increase in its basal width and its length, lead to a stage where the second scale possesses massive lateral wings, which are gradually enclosing the primordia of the third and fourth scales (figs. 9 and 10). Following this stage, the further history of development of the second scale is essentially like that already described for the first scale, with the difference that the apex usually remains more obtuse and less prominent (cf. figs. 7 and 10).

The papillate primordium of the third scale appears at the side of the relatively broad growing point during or just preceding the determinate stage in development of the second scale (fig. 9). During its rapid increase in size, dorsal hairs develop rapidly on its median-central and apical regions, as is the case in the young primordia of the first and second scales. When the determinate stage has been reached, the primordium of the third scale is very distinctive in form. Viewed dorsally, it appears as a relatively massive, broad-based initial which is obviously experiencing rapid apical growth. From this aspect, its left basal margin is seen to be growing in front of the primordium of the fourth scale (fig. 10). A ventral aspect of the primordium of the third scale at the same ontogenetic stage emphasizes its thick, broad-based character and shows the slightly concave nature of its ventral surface resulting from the active ventral-marginal growth which has already begun (Pl. III, fig. 11). Up to and including the determinate stage in development, the primordium of the third scale grows more rapidly in basal width than in height, the ratios computed from measurements of six different initials varying from .49–.8. Following the determinate stage in its ontogeny (fig. 11) the primordium of the third scale, as a result of active growth on its ventral margins, together with a relatively slow increase in its basal width as compared with its height (the ratio, as in the first and second scales, soon exceeds 1), rapidly acquires a conical form (fig. 12). In this ontogenetic stage, the massive lateral wings, which extend nearly to its apex, are quite distinct from the slowly thickening median-central region of the scale, and soon enclose the primordia of the fourth and fifth scales.

The main features common to the morphogenetic period of development of the first three bud scales may be summarized as follows. The papillate

primordium, as a result of rapid increase in basal width, height, and thickness, reaches the determinate stage during which active growth at the ventral margins and apical extension are initiated. Up to and including the determinate stage, the ratio of height to basal width in the primordia varies but is invariably less than unity. Following the determinate stage, wing formation proceeds actively and uniformily, and eventually the scales acquire the form of broad-based, conical structures with a definite equitant aestivation. Reference to table 1 indicates that the completion of the critical developmental phases of the first three scales coincides with the periods of swelling and internodal elongation of the main shoot. Aside from histological specializations, such as the formation of colleters and very numerous dorsal hairs and the maturation of the vein system, the remainder of the development of the outer scales is essentially an increase in size of the form shown in figures 7 and 10, Plate II and figure 12, Plate III, and leads directly to the ovate-acute, vaginate form of these organs at maturity (cf. Foster, 1931 *b*, Pl. LXI, figs. 4, 5, and 6).

The Inner Bud Scales (4–7 Inclusive)

The primordium of the fourth scale is papillate in form when it first appears at the growing point. As a result of rapid increase in size (fig. 11), it soon reaches the determinate stage where it has the form of a broad-based, thickish initial. When seen dorsally in this ontogenetic stage, the primordium of the fourth scale appears to possess a slightly narrower basal width than the third scale during the latter's determinate stage (cf. fig. 10, Pl. II and fig. 12, Pl. III). This situation seems to be explained by the fact that although the ratio of height to basal width in the primordium of the fourth scale during its earliest growth varies from .5–.8 (five initials measured), when the determinate stage has been reached, the ratios as computed from measurements on thirteen different initials ranged from .6–1.01 These figures illustrate an important feature in the early developmenta. history of the inner scales, *viz:* that following the papillate stage, the basal width of the primordia increases at a relatively slower rate than is the case with the initials of the first three bud scales. After the primordium of the fourth scale has reached the determinate stage, its apex extends rapidly and its ventral margins begin active growth (fig. 13). Following the stage just described is a period during which the scale increases relatively slowly in basal width, grows more actively in height, and acquires massive lateral wings (fig. 14). A significant feature of this ontogenetic period of the fourth scale is the fact that the lateral wings tend to grow most rapidly at a point a short distance above the scale base. Furthermore, there is a pronounced tendency for the two wings to develop unequally, leading to a more or less definite asymmetry. An interesting aspect of this asymmetrical growth of the scale-wings lies in the fact that the more rapidly growing wing is slightly constricted near its base and is directly in contact, at the region of constriction, with the youngest foliar primordium. The developmental features

just described as characteristic of the fourth scale become increasingly evident in the fifth, sixth, and seventh scales and are clearly associated with the obovate or spatulate (more or less) asymmetrical form of these organs at maturity.

Reference to table 1 shows that the morphogenetic period of development of the inner scales occurs at a time when the new terminal bud is fully exposed at the tip of the shoot. It should be appreciated, however, that the primordia of the fifth, sixth, and seventh scales pursue their development within a protected "chamber" formed by the closely overlapping wings of the outer bud scales. Thus, in a biological sense, the outer bud scales early assume a protective rôle for the remaining scale primordia.

The tiny papillate primordium of the fifth scale appears at the growing point during the determinate stage in ontogeny of the fourth scale (figs. 12 and 13). During the early phases in its ontogeny, the basal width of the primordium of the fifth scale grows at a faster rate than its height, measurements on sixteen initials giving ratios which extended from .6–.8. As the primordium approaches the determinate stage, however, the rate of growth in its height begins to increase slowly as shown by the fact that the ratios obtained from measurements on nine different initials ranged from .7–1, the latter figure being obtained in two instances. When viewed laterally at this developmental stage, the primordium of the fifth scale is a relatively thick, broad-based structure in which extremely active apical extension has already begun (fig. 15). This early appearance of vigorous apical growth is characteristic of the primordia of the fifth, sixth, and seventh scales and stands in contrast to the situation in the primordia of the outer scales at a comparable developmental stage (cf. fig. 9, Pl. II). During and following the ontogenetic period of the fifth scale portrayed in figure 15, rapid growth begins on the ventral margins of the primordium, giving rise to rapidly growing lateral wings. A study of a later ontogenetic stage shows that the most active growth of the wings occurs about midway between the rather acute apex and the slowly broadening base of the scale (fig. 16). This figure likewise illustrates the early asymmetrical growth of the scale-wings and the contact of the primordium of the seventh scale with the constricted region of the left scale-wing. When viewed ventrally at this ontogenetic stage, the wings of the fifth scale appear as two rather asymmetrical flaps which are obviously growing most actively above the broadened scale-base (Pl. IV, fig. 28). This mode of development of the scale-wings stands in marked contrast to the more uniform growth of the wings of the outer scales (cf. figs. 7 and 10, Pl. II and fig. 12, Pl. III, with fig. 16, Pl. III).

During the early phases of growth of the sixth scale, the basal width of the primordium increases at a faster rate than its height (figs. 14 and 15). Ratios computed from measurements on twelve different initials during this period of ontogeny ranged from .5–.8. When viewed ventrally just before the determinate stage is reached, the primordium of the sixth scale appears

as a thick, broad-based, dorsally convex and ventrally slightly concave structure, showing little or no evidence of marginal growth (fig. 17). The determinate stage, as usual, is characterized by rapid apical extension and the initiation of growth on the ventral margins of the primordium (fig. 16). During this ontogenetic period, the rate of growth in length begins to exceed the rate of basal widening, ratios derived from measurements on five initials ranging from .8–1.3. These ratios again emphasize the important difference in rates of growth occurring during the determinate period in ontogeny of the outer and inner scales. Following the stage depicted in figure 16, the sixth scale grows slowly in basal width, extends rapidly in length, and acquires characteristic asymmetrical wings (fig. 18). At this stage, the fifth and sixth scales are developing in essentially the same manner (figs. 16 and 18).

The primordium of the seventh scale appears as a papilla at the side of the growing point (fig. 15). Measurements of seven initials during their early phase of growth (figs. 16 and 17) showed that a marked tendency exists for the rate of basal widening to exceed the growth in height, even before the determinate stage has been reached, the ratios extending from .6–1.1. When viewed laterally before marginal growth has commenced, the primordium of the seventh scale resembles the primordium of the sixth scale at a comparable developmental stage, except that its apex is slightly more acute (cf. figs. 17 and 18). A lateral-ventral aspect of the primordium of the seventh scale, at about the same ontogenetic stage, illustrates the pronounced apical extension which is occurring (fig. 19). During the determinate stage, the primordium of the seventh scale increases its length at a rapid rate, ratios obtained from measurements of four initials ranging from .8–1.5. The actively developing lateral wings, resulting from the growth initiated at the ventral margins, become asymmetrical at an early period and are greatest in extent above the slowly-broadening scale base (fig. 20). Further growth of the seventh scale leads to the more or less obovate-acute form of this organ in the winter bud (cf. Foster, 1931 *b*, Pl. LXI, fig. 9).

The important aspects of the morphogenetic period of development of the inner bud scales may now be summarized briefly. During the earliest phases of ontogeny, there is a marked tendency, which gradually increases in the successive scales, for the ratio between growth in height and growth in basal width to approach and finally to exceed unity. The significance of this mode of early growth is that when the determinate stage is reached, the primordia are definitely longer than broad. Apparently in correlation with their shape, wing-formation becomes most active at a point midway between the apex and the slowly-broadening bases of the scale. As a result of this type of growth, a more or less clear demarcation may be drawn, at a relatively early developmental stage, between a short basal region, which is experiencing relatively little marginal extension, and an upper region which is forming massive lateral wings (figs. 14, 16, 18, and 20). This configuration is maintained during the subsequent increase in size of the inner scales

and leads to the more or less definitely obovate form of these organs in the winter bud (Foster, 1931 *b*, Pl. LXI, figs. 7 and 8). During the expansion of the bud in spring, active elongation occurs in the narrowly-winged basal region of the inner scales and results in the obovate or spatulate form of these organs at maturity (Foster, 1931 *b*, Pls. LXII and LXIV).

Reference to table 1 shows that the period of scale formation ends with the appearance of the primordium of the seventh scale on April 18. Although marked by little appreciable change in its appearance, the growing point, from this period onward until about May 13, forms the primordia of the foliage leaves, which pursue their critical ontogenetic stages within a "chamber" formed by the rapidly enlarging bud scales.

The Foliage Leaves

The primordium of the first foliage leaf appears as a papillate swelling on the side of the growing point just prior to the determinate stage of development of the sixth scale (fig. 17). In its earliest stages, this foliage-leaf primordium, save for its position in the foliar cycle, gives no outward indication of its destiny. As it enlarges in size, however, it is soon evident that growth in height is proceeding at a much faster rate than growth in basal width (fig. 19). Ratios computed from measurements of eighteen foliage-leaf primordia during the early ontogenetic period ranged from 1.1–1.5. This relatively rapid growth in length accompanied by much slower basal widening continues and leads to the determinate stage in ontogeny where the primordium appears as a narrow-based, acute, semi-terete structure with a convex dorsal surface and a nearly plane ventral surface (Pl. IV, fig. 21). One of the specific characteristics of a foliage-leaf primordium in the determinate stage is the rapid development of hairs on the lower region of its ventral surface. Insignificant as this feature may seem, it clearly distinguishes a foliage-leaf primordium from a bud-scale primordium even before the more characteristic and divergent methods of growth have begun in these initials (cf. figs. 19 and 20). Following the ontogenetic stage depicted in figure 20, there occurs a most critical morphogenetic type of growth which is fundamentally responsible for the striking divergence between bud scale and foliage leaf in the black hickory. Beginning first as a slight swelling near the base of its ventral surface, a double series of papillae appear in acropetal succession. These papillae, which begin to enlarge rapidly prove to be the primordia of the lateral leaflets; the acuminate terminal portion of the foliage-leaf primordium above the youngest pair of lateral leaflet primordia is destined to develop into the terminal leaflet (fig. 22). As a result of the acropetal method of development of the lateral leaflet primordia, the foliage-leaf initial is early demarcated into a rapidly growing lamina and a short, thickened, slowly-broadening basal region.[5] The latter is destined to give rise to the leaf base and the petiole in a manner to be described presently.

[5] Langdon (1931, p. 286) reports an acropetal order of development of the upper lateral leaflet primordia in *Carya cordiformis*.

During the period of lateral leaflet formation, the hairs on both the dorsal and ventral surfaces of the foliage-leaf initial increase in number and length; eventually, the young foliage leaf is densely pubescent and great difficulty is consequently experienced in following its later developmental stages.

In the previous paper in this series (Foster, 1931 *b*, table I), I have emphasized the variability in the number of pairs of lateral leaflets present in the successive leaves of a given shoot. It was shown that the first foliage leaf tends to have seven leaflets and that a progressive (although somewhat erratic) reduction in lateral leaflets occurs in successive leaves. The general appearance of the first foliage leaf, as shown in figure 22, suggests that no more lateral leaflet primordia would have appeared. The mature foliage leaf would have thus had five leaflets.

The early history of development of the three remaining foliage leaves is similar in all essentials to the ontogeny of the first foliage leaf. The papillate primordium of the second foliage leaf appears at the growing point during the determinate stage of the first foliage leaf (figs. 20 and 21). A lateral view of this primordium at a much later stage illustrates clearly the initiation of lateral leaflet development by the appearance of a swelling near the base of its ventral surface (fig. 22). When viewed ventrally at a later developmental stage, the second foliage leaf may be seen to have formed two pairs of lateral leaflet primordia; note the rapidly developing hairs on the slightly concave ventral surface of the primordium (fig. 24).

The primordium of the third foliage leaf appears just prior to the initiation of lateral leaflet primordia in the second foliage leaf (fig. 22). It is of interest to note its contact with one edge of the first foliage leaf, recalling the situation already described under the ontogeny of the bud scales (cf. figs. 20 and 22). When viewed ventrally at a much later developmental stage, the third foliage leaf is seen to have developed three pairs of lateral leaflet primordia, the decreasing size of each successive pair clearly indicating their acropetal order of formation (fig. 25). During this ontogenetic stage of the third foliage leaf, the primordium of the fourth foliage leaf appears at the growing point. Its further development is similar to that of the preceding foliage leaves and requires no further description.

As is shown in figure 23, the cluster of rapidly developing foliage leaves is soon more or less completely enclosed by the enlarging wings of the seventh bud scale. This figure likewise serves to illustrate the utter and abrupt divergence in the organization and mode of growth of the last scale and the foliage leaf. The further development of the foliage leaves consists in a gradual increase in their size accompanied by a further specialization in their laminar and basal regions; for convenience we may describe first of all the further development of the lamina.

The lateral leaflet primordia, which at first are papillate in form (fig. 25), soon begin to elongate rapidly and finally appear as finger-shaped structures. The terminal leaflet primordium, as a result of similar growth processes,

attains a like configuration. Beginning first in the terminal leaflet, and proceeding basipetally in the successive pairs of lateral leaflets, there appear on the ventral surface of each leaflet, two delicate meristematic weals or ridges which represent the first indication of blade development (fig. 26). It should thus be evident that the thickened apically elongating portion of each leaflet corresponds to its future midrib.[6] The growth of the ventral wings of each leaflet proceeds rapidly and the length of the wings continues to increase in the direction of elongation of the leaflet. When the leaflet-wings have reached a noticeable width, the ventral wing of each lateral leaflet (*i.e.* the wing nearest the center of the bud) begins to grow over and enclose the inner wings; a continuation of this unequal growth leads eventually to the convolute-involute vernation of the leaflets which I have previously described (Foster, 1931 *b*, p. 870). During the early period of blade development, a series of transverse ribs begin to appear in acropetal succession in the leaflet wings (fig. 27). These ridges are the early stages in development of the main lateral veins of the leaflet and their marginal extensions correspond to the point of origin of the main serrations of the adult leaflet (cf. Foster, 1931 *b*, text fig. 2). In subsequent developmental stages, the lateral ribs of the leaflet wings become separated by a type of intercalary growth which is probably due to the activity of a "Plattenmeristem." [7] As the main lateral ribs become gradually separated in this way, secondary veins, appearing as smaller ribs, develop from their distal ends and eventually give rise to the forked or trifurcated condition of the main lateral veins of the adult leaflet.

During the period of active growth and blade-formation in the leaflets, the basal region of the young foliage leaf slowly broadens and elongates and becomes noticeably thickened (figs. 26 and 27). In the winter bud, it is usually possible to distinguish, more or less clearly, the leaf-base and the petiole, because of greater breadth of the former.

The first two or three foliage leaves have attained a considerable degree of morphological complexity when further growth of the organs of the bud is terminated by the approach of cold weather. In the winter bud, the foliage leaf is seen to consist of a broadened leaf base, a short petiole, and a well-developed lamina (cf. Foster, 1931 *b*, Pl. LXI, fig. 9). During the expansion of the bud in the spring, active intercalary growth occurs between the lamina and the leaf base leading to the elongated mature petiole, the leaflets develop and expand in an acropetal direction and a rachis is formed between the lower pairs of lateral leaflets, and the adult foliage leaf appears (cf. Foster, 1931 *b*, text fig. 2).

[6] Langdon (1931, p. 286 and Pl. II, fig. 21) reports a similar mode of development of the blade and midrib of the leaflets in *Carya cordiformis*.

[7] Cf. Schüepp (1926, p. 18).

Discussion

The results of this investigation may be discussed most advantageously in relation to two main problems, *viz:* (1) organization and form of the adult cataphylls and foliage leaf and (2) the concept of the "leaf primordium."

In the previous paper of this series, the cataphylls were interpreted, on the basis of their adult form and venation, as foliar organs with a simple morphological organization which precludes any direct homology being drawn between them and the "elements" of the adult foliage leaf. Evidence was presented to support the idea that the fundamental architecture of the cataphyll is that of a foliar organ possessing a more or less distinct axial region (or "phyllopodium") flanked by two thin, often asymmetrical wings. How do these ideas appear when re-examined in the light of ontogenetic facts?

In the first place it has been shown that during the critical morphogenetic phase in ontogeny, the relatively simple form of the cataphyll and the highly segmented character of the foliage leaf are determined. In the case of the scale, the determinative ontogenetic process consists in the initiation of active growth on the ventral margins of the primordium, resulting in the rapid formation of wings. The foliage-leaf primordium, in contrast, forms in acropetal succession a double series of papillate lateral leaflet primordia on its ventral margins. Associated with the acropetal order of appearance of lateral leaflet primordia, the primordium of a foliage leaf is early segmented into two discrete regions *viz:* (1) a rapidly growing "lamina" and (2) a slowly developing basal region destined to give rise, by subsequent processes of marginal, radial, and intercalary growth, to the leaf base and the petiole. The evidence from ontogeny thus clearly supports the idea already derived from a study of adult structure, that any attempt to homologize the scale with the "morphological elements" of the foliage leaf rests upon an insecure foundation. It is true that during the early development of the inner bud scales, a more or less evident basal region becomes distinct, as a result of less active marginal growth, from the upper, massively-winged region of the scale and that intercalary growth in this basal region during bud expansion gives rise to the stalk-like portion of the scale. However, to conclude from this fact that a transverse demarcation should be drawn between a "petiole" and a "lamina" region in the adult cataphyll is in my opinion gratuitous. Bower (1889, p. 248) expresses a view which seems particularly cogent in this connection when he states: "While we note that a distinction of petiole and lamina, as parts having usually a definite relation of form and position, is applicable to a large proportion of leaves, let us beware of assuming that the parts of *all* leaves, however peculiar their form, are referable to such a type."

The question now arises as to the interpretation of the "apical laminar region" or "terminal lobe" which, as I have already shown (Foster, 1931 *b*), occasionally appears in the uppermost cataphylls of certain shoots. Owing

to the sporadic occurrence of this "terminal lobe" it has not been possible to follow its development with any accuracy. The ontogeny of the inner bud scales, however, suggests that the more or less discrete and narrowly winged apical region of the primordium may undergo a specific process of internal differentiation similar to that followed by the terminal leaflet of the foliage leaf. That this may be the case is suggested by the dark green color and the presence of weak reticulate venation in the terminal lobe in many cases (Foster, 1931 *b*, Pl. LXIV). The nature and time of appearance of the growth processes leading to the formation of a terminal lobe, however, can be determined only by a careful examination of the internal development of the scale, a matter reserved for future investigation.

Ontogenetic facts seem to support the view already expressed that the cataphyll fundamentally consists of an axial region flanked by lateral often asymmetrical wings. The distinction between the wings and the axial region is particularly obvious during the early stages of ontogeny (Pl. IV, fig. 28). During the later phases of development, a gradual increase in thickness appears throughout the scale making the distinction less obvious. At maturity, however, the appearance of a more definite dorsal rib and especially the presence of a pinnately-branching midvein in the median central region of the cataphyll further suggest that the scale possesses an alate construction. From this standpoint the median central region of the scale may be regarded as the phyllopodium or foliar axis upon which, in varying degrees, thin lateral outgrowths or wings are developed. If this interpretation is sound, it follows that the basic morphological distinction between the cataphyll and the foliage leaf does not consist primarily in the fact that the latter, at maturity, possesses certain formal elements, *viz:* the leaf base, petiole, and lamina since the distinction of these elements, as I have shown, depends to a large extent upon secondary processes of intercalary growth.[8] On the contrary, the basis for the morphological divergence between scale and foliage leaf is found in the determinate stage in ontogeny of these organs where the phyllopodium of the scale develops marginal outgrowths or wings while the axis of the foliage leaf forms "branches," *i.e.* lateral leaflets.

Cook's (1916, 1923) interpretation of hickory cataphylls as "sheaths" has already been rejected for the black hickory on evidence derived from adult form and venation. Ontogenetic facts likewise furnish no evidence for its acceptance but show, on the contrary, that the cataphyll is a unit foliar organ which, although invariably simple and often vaginate in form at maturity, is clearly not homologous with a sheath. The present study shows that the sheath-like character of the outer bud scales results from three primary ontogenetic processes, *viz:* (1) the rather uniform development of wings, (2) the maintenance of the early vaginate form during the subsequent period of enlargement, and (3) the failure of these scales to become

[8] This is clearly the case in the development of the elongated form of the petiole and in the late appearance of the rachis.

noticeably accrescent during bud expansion. The obovate or spatulate form of the inner scales is likewise capable of a developmental interpretation. During the early ontogeny of these organs, wing development is not uniform but is most active at a point midway between apex and base; intercalary extension in the narrowly-winged basal region of the scale during bud expansion inevitably leads to the obovate or spatulate type of configuration. Unfortunately, it has not been possible to discover the growth factors responsible for the emarginate apex of the scale or to investigate the causes for the extraordinary polymorphism of the inner cataphylls. This phase of the problem will always present considerable difficulties in view of the erratic occurrence of the various form types (cf. Foster, 1931 *b*).

As I pointed out in an earlier paper (Foster, 1928, p. 147), one of the most fundamental concepts with which the ontogeneticist must constantly deal is that of the "leaf primordium." Definitions are usually of temporary value and require continual modifications as our knowledge increases but perhaps a brief discussion of the bearing of recent work on the meaning and usage of the expression "leaf primordium" may be of value.

The most recent and able discussion of the concept of "leaf primordium" has been given by Schüepp (1929, pp. 796–800), who points out very clearly that nearly every morphological and developmental-physiological analysis of form is usually reducible to the viewpoint of either preformation or epigenesis. According to the theory of preformation, one must regard the adult form of a leaf as being implicit and determined in the specific, unalterable nature of the primordium. In contrast, the epigenetic viewpoint, which was so earnestly championed by Klebs (1903), considers form the result of inner and outer factors which are constantly operative during the development of the leaf primordium, each developmental phase being more or less specific in itself and *conditioning*, to some extent at least, the stage following it.[9] Schüepp then points out the significant fact that Goebel's "Transformation" theory, to which I have raised both theoretical and practical objections (Foster, 1928, p. 148 and 1929, pp. 482–485), attempts to combine and reconcile the opposed viewpoints of preformation and epigenesis by assuming that the "foliar primordium" is *preformed* as a foliage-leaf primordium and subsequently, as a result of changed conditions obtaining during its development, becomes *epigenetically* "transformed," for example to a bud scale. Schüepp's own studies on *Acer Pseudoplatanus*, however, led him to conclude that at least in the case of the inner bud scales, the "transformation" can not be demonstrated either developmentally or experimentally. In an effort to avoid the difficulties inherent in Goebel's theory of "arrested formations," Schüepp suggests that we may consider a working plan ("Arbeitsplan") to be present in the foliar primordium, by means of which the position, time, seriation, and duration of the individual growth processes are deter-

[9] Cf. Reinke (1901, pp. 359–385) for an excellent discussion of epigenesis in plants, with special reference to shoot development.

mined. The *degree* to which these complex growth processes occur is determined, in Schüepp's view, by the inner and outer conditions under which the plant grows, the former being regarded as "the living, autonomous causes of changes." Schüepp then outlines the "working plan" of a developing leaf in *Acer* and attempts to show how it may be applied in an interpretation of the divergent ontogeny of bud scale and foliage leaf. He concludes that the notion of the "real transformation" of the visible primordium is replaced, in his theory, by a "metamorphosis" or change of the "working plan."

Schüepp's viewpoint is clearly a "plastic" one and perhaps for that reason preferable to any attempt at a more rigid definition of the term "leaf primordium." In a previous paper (Foster, 1929, p. 484) I concluded that in *Aesculus Hippocastanum* "the developmental history of the scale is a continuous and determinate process." This conclusion, which was based upon a consideration of the difference in growth rates between the leaflet primordia and the hypopodium of the scale and the foliage leaf, seems even more justifiable in the case of the black hickory where cataphyll and foliage leaf diverge at the very earliest period in ontogeny. To interpret the ontogenetic facts otherwise, one would have to assume, as Goebel (1928, p. 429) does in similar cases, that the "transformation" has occurred so early that it is no longer visible but can only be demonstrated on comparative grounds. Such an assumption in my opinion would be valueless and certainly of no aid in understanding the growth processes involved. The ontogeny of the upper transitional forms in the black hickory, during which a rudimentary laminar primordium may be laid down, seems comparable to the development of the bud scales in *Aesculus Hippocastanum*, and in no direct sense is an indication of their origin from foliage-leaf primordia.

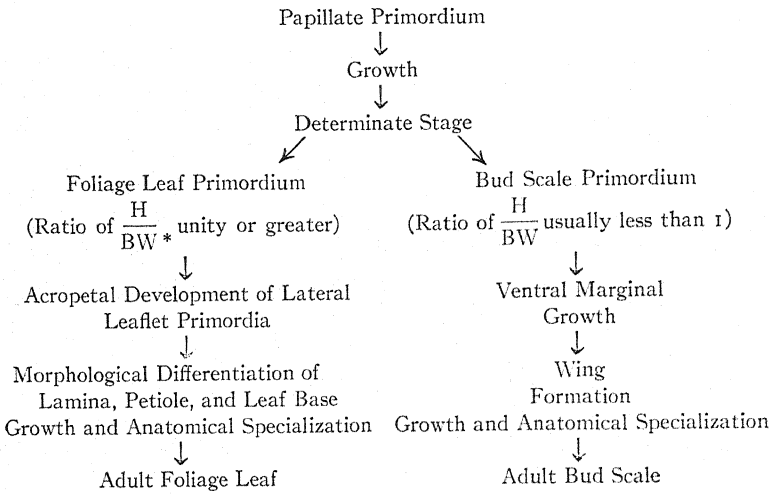
The following scheme (p. 94) may serve to emphasize my viewpoint as to foliar ontogeny in the black hickory. It will be obvious, from what has been said above, that the terms "scale primordium" and "foliage-leaf primordium" are used to indicate that in the developing foliar primordium, morphogenetic growth processes are appearing which, if persistent and followed by other "causally" related processes, inevitably lead to the adult cataphyll and foliage leaf.

Summary

1. The annual foliar cycle on spur shoots of the black hickory consists, in the average case, of two upper transitional forms, seven bud scales, and four foliage leaves. A table is presented comparing the periods of initiation of the successive foliar organs of the new terminal bud with the general processes of growth in the main shoot.

2. The primordia of the upper transitional forms are present at the growing point of the winter bud and resume their growth during the period of bud swelling in the spring. The laminar primordium of the outer transitional form grows slowly whilst its basal portion rapidly develops massive wings. The inner transitional develops to a simple winged organ with a prominent apical pointlet.

SCHEME OF BUD SCALE AND FOLIAGE LEAF ONTOGENY



* H = height; BW = basal width.

3. The primordia of the scales of the new terminal bud are laid down during the elongation of the main shoot. The critical morphogenetic phase in their development consists in the initiation of ventral-marginal growth with the resultant formation of wings.

4. Foliage-leaf primordia directly follow the initiation of the last bud scale. Their critical morphogenetic growth consists in the acropetal development, on their ventral margins, of the primordia of the lateral leaflets. Blade formation is initiated by the development of two meristematic ridges or weals on the ventral surfaces of the leaflets and is followed by the acropetal succession of marginal teeth and the main lateral veins in the resulting wing-like extensions. Varying degrees of marginal, radial, and intercalary growth in the basal region of the foliage leaf lead to the distinction between the leaf base and the petiole. The rachis appears last as the result of intercalary growth between the lower pairs of lateral leaflet primordia.

5. The plastochron of the bud scale is shorter than that of the foliage leaf.

6. Ontogenetic facts indicate that the basic morphological divergence between cataphyll and foliage leaf is determined during their morphogenetic phase of development, the critical growth factors being, respectively, wing formation and the acropetal formation of lateral leaflet primordia.

7. Evidence derived from ontogeny supports the morphological interpretation previously advanced that the fundamental architecture of the cataphyll is that of a simple foliar organ possessing an axial region flanked by lateral often asymmetrical wings.

8. A brief discussion is given of the bearing of recent work on the concept

of "leaf primordium," and is followed by a diagram indicating the early determinate nature of scale and foliage-leaf ontogeny in this species.

I am deeply grateful to my wife, Helen Vincent Foster, for her painstaking and skillful preparation of the illustrations used in this paper and for her assistance in proof-reading. My thanks are due also to Dr. Paul B. Sears for his constructive criticism of several points taken up in this paper.

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EXPLANATION OF PLATES

All of the figures in the three following plates, with the exception of FIGS. 26–28 represent the critical morphogenetic stages in development of the successive foliar organs of the terminal bud of the spur shoot during 1930. In general, the figures are arranged in an order corresponding to the successive appearance of the foliar primordia at the growing point during a complete foliar cycle, *i.e.* one beginning with the condition found in the winter bud and ending with the initiation of the last foliage-leaf primordium. Following the description of each figure there appear in parentheses the significant dimensions of the larger primordia, expressed in terms of basal width × height; and the actual magnification of the figure in question.

PLATE II

FIGS. 1 and 2. Lateral-ventral aspects of the primordia of the upper transitional forms at the growing point of two different buds, February 19 (trichomes not shown). FIG. 1, the larger of the two primordia shows the origin of the apical pointlet characteristic of these organs and presumably would have remained unsegmented throughout ontogeny (larger: 300 μ long; smaller: 178.75 μ × 143 μ. × 83). FIG. 2. The larger of the two primordia has formed a pair of lateral leaflet primordia, a common situation; the smaller probably would have remained unsegmented (larger: 321.75 μ long; smaller: 214.5 μ × 143 μ. × 87).

FIG. 3. Lateral aspect of the primordia of the upper transitional forms which are enclosing the primordium of the first scale, March 22 (trichomes not shown). This figure represents a developmental stage following that shown in FIG. 2. The primordia of both transitional forms have increased greatly in basal width and are developing massive lateral wings. Note the ventral weals on the median and one lateral leaflet primordium of the outer of the two transitional forms (first trans. form: 715 μ long; second trans. form: 536.25 μ × 393.25 μ; first scale: 250.25 μ × 143 μ. × 65).

FIG. 4. Ventral and dorsal aspects, respectively, of the primordia of the second transitional form and the first scale of the bud shown in FIG. 3 with the first transitional form removed to show details (trichomes not shown). Note the rapidly developing wings of the transitional form which terminate just below its pointed apex. At the right of the primordium of the first scale, which as yet shows no marginal growth, is found the relatively broad growing point (× 109).

FIG. 5. Ventral-lateral aspects of the second transitional form and the primordium of the first scale, and dorsal aspect of the primordium of the second scale at the growing point, March 22. A slightly older stage than that shown in FIG. 4. Marginal growth and apical extension have begun in the primordium of the first scale while the second scale appears as a rounded protuberance at the side of the growing point (trans. form: 393.25 μ × 375.5 μ; first scale: 250.25 μ × 143 μ. × 98).

FIG. 6. Ventral-lateral aspects, greatly enlarged, of the primordia of the first two scales at the growing point, April 12. The primordium of the first scale, which is developing dorsal hairs, is broadly ovate, obtuse, and beginning to show active marginal growth. The primordium of the second scale is papillate (first sc.: 214.5 μ × 178.75 μ. × 112).

FIG. 7. Ventral aspect of the first scale, dorsal-lateral aspects of the primordia of the second and third scales, April 12. An important developmental stage following that shown in FIG. 6. Note the prominent wings of the first scale which are beginning to cover the younger scale primordia. The primordium of the second scale exhibits a prominent apical constriction which is characteristic of the early phases of scale ontogeny (first sc.: 500 μ long; second sc.: 286 μ × 214.5 μ. × 66).

FIG. 8. Ventral and dorsal aspects, respectively, of the first and second scales, April 12. An older stage than in the preceding figure, illustrating the origin of the characteristic early equitant aestivation of the lower cataphylls. Compare Foster, 1931 *b*, text fig. 1. (First sc.: 825 μ long. × 36).

FIG. 9. Ventral-lateral aspects, greatly enlarged, of the primordia of the second and third scales at the growing point, April 12. The primordium of the second scale is broad-based and is beginning to grow actively at its apex and margins. Notice the early appearance of dorsal hairs on the tiny primordium of the third scale. This developmental stage of the second and third scales is comparable to that shown, at less magnification, in FIG. 7 (second sc.: 286 μ × 214.5 μ. × 93).

FIG. 10. Ventral aspect of the second scale; dorsal and lateral aspects, respectively, of the primordia of the third and fourth scales, April 12. A developmental stage following that shown in FIG. 9. Characteristic features of the second scale are its obtuse apex and uniformly developing wings which are growing over the younger scale primordia. Note that the left edge of the primordium of the third scale stands in front of the primordium of the fourth scale (second sc.: 464.75 μ × 429 μ; third sc.: 286 μ × 214.5 μ. × 65).

PLATE III

FIG. 11. Ventral-lateral aspects, greatly enlarged, of the primordia of the third and fourth scales at the growing point, April 12. About the same developmental stage of the third and fourth scales as shown in FIG. 10. The primordium of the third scale has the relatively thick, broad-based character of all the lower scale primordia; it is beginning to exhibit marginal growth (third sc.: 250.25 μ × 214.5 μ. × 93).

FIG. 12. Ventral aspect of the third scale; dorsal and lateral aspects, respectively, of the primordia of the fourth and fifth scales, April 12. A developmental stage following that shown in FIG. 11. Note the general similarity between the second and third scales at comparable ontogenetic stages (cf. fig. 10.) The primordium of the fourth scale already shows the initiation of apical constriction. A portion of the growing point is visible between the primordia of the fourth and fifth scales (third sc.: 357.5 μ × 286 μ; fourth sc.: 214.5 μ × 143 μ. × 76).

FIG. 13. Ventral-lateral and lateral aspects, respectively, of the primordia of the fourth and fifth scales at the growing point, April 12. A magnified view of the same developmental stage of the fourth and fifth scales as shown in Fig. 12. Note the broad-based character of the primordium of the fourth scale, upon the dorsal surface of which numerous hairs are beginning to appear (fourth sc.: 178.75 μ × 143 μ. × 160).

FIG. 14. Lateral-ventral aspect of the fourth scale, lateral and dorsal aspects, respectively, of the primordia of the fifth and sixth scales, April 15. A developmental stage following that shown in FIG. 13. Note particularly the more rapid development of the wings of the fourth scale above its base; this type of growth leads to the obovate form of the adult cataphyll. The primordium of the fifth scale shows a slightly different form from that of the preceding scales at a similar developmental stage (fourth sc.: 357.5 μ × 464.75 μ; fifth sc.: 214.5 μ × 178.75 μ. × 79).

FIG. 15. Ventral-lateral aspects of the primordia of the fifth and sixth scales, and dorsal aspect of the primordium of the seventh scale, all greatly enlarged, at the growing point, April 15. A slightly older stage of these organs than that shown in FIG. 14. Although growing rapidly in basal width, the primordium of the fifth and sixth scales show no indication of wing formation at their margins. The primordium of the seventh scale appears as a papilla on the side of the growing point (fifth sc.: 250.25 μ × 178.75 μ. × 150).

FIG. 16. Ventral-lateral aspect of the fifth scale; lateral and dorsal aspects, respectively, of the primordia of the sixth and seventh scales, April 18. A developmental stage following that shown in FIG. 15. Note that due to the more rapid development of the wings above its base, the fifth scale only partially encloses the younger scale primordia (contrast with FIG. 12). In the fifth and succeeding scales, one wing is definitely constricted just above the base and the youngest foliar primordium (in this case the primordium of the seventh scale) always stands next to this constriction. The primordium of the sixth scale exhibits the ovate-acute form characteristic of the primordia of the inner cataphylls (fifth sc.: 429 μ × 500.5 μ; sixth sc.: 286 × 250.25 μ. × 68).

FIG. 17. Ventral-lateral, lateral, and dorsal aspects, respectively, greatly enlarged, of the primordia of the sixth and seventh scales and first foliage leaf at the growing point, April 18. This figure exhibits a slightly younger developmental stage of the primordia of the fifth and sixth scales than that shown in FIG. 16. Marginal growth has not yet begun in the primordium of the sixth scale. The primordium of the foliage leaf is merely a rounded papilla at the side of the growing point (sixth sc.: 178.75 μ × 143 μ. × 140).

FIG. 18. Lateral-ventral aspect of the sixth scale; lateral and dorsal aspects, respectively, of the primordia of the seventh scale and the first foliage leaf, April 21. A developmental stage following that shown in FIG. 17. The sixth scale, at this stage, shows features similar to those of the fifth scale in a corresponding stage of ontogeny (cf. FIG. 16). The primordium of the seventh scale shows rapid and characteristic elongation and the early appearance of dorsal hairs although marginal growth has not yet begun. The primordium of the foliage leaf shows no specific characteristics at this stage (sixth sc.: 357.5 μ × 393.25 μ; seventh sc.: 250.25 μ × 178.75 μ. × 78).

FIG. 19. Lateral-ventral aspects, greatly enlarged, of the primordia of the seventh scale and first foliage leaf at the growing point, April 21. Approximately the same developmental stage of the primordia of the seventh scale and first foliage leaf as shown in FIG. 18. Note particularly the ovate-acuminate form of the primordium of the seventh scale at a developmental stage when marginal growth has not yet begun, a condition unlike that occurring at a corresponding stage in ontogeny of the lower cataphylls (seventh sc.: 214.5 μ × 178.75 μ. × 140).

FIG. 20. Lateral-ventral aspect of the seventh scale; lateral and dorsal aspects, respectively, of the primordia of the first two foliage leaves, April 20. A developmental stage following that shown in FIG. 19. Aside from its more pointed apex, the seventh scale at this developmental stage shows the same characteristic features exhibited by the fifth and sixth scales at a comparable stage in ontogeny (cf. FIGS. 16 and 18). The primordium of the first foliage leaf, although unsegmented, exhibits a specific character in the development of ventral hairs, a condition never found during young stages of scale development (seventh sc.: 321.75 μ × 500.5 μ; first foliage leaf: 178.75 μ × 250.25 μ. × 74).

PLATE IV

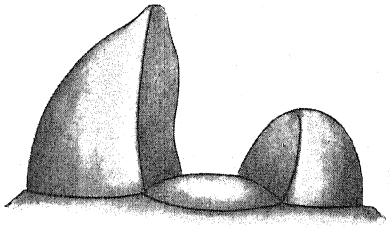
FIG. 21. Lateral-ventral and lateral aspects, respectively, of the primordia of the first two foliage leaves at the growing point, April 23. A slightly younger stage than that shown in FIG. 20, in that ventral hairs have not yet appeared on the larger of the two primordia (first foliage-leaf primordium: 178.75 μ × 250.25 μ. × 108).

FIG. 22. Lateral aspect of the first foliage leaf; lateral and dorsal aspects, respectively, of the primordia of the second and third foliage leaves, April 30. A significant developmental stage following that shown in FIG. 21. Note the prominent dorsal and ventral hairs on the first foliage leaf which has formed, in acropetal succession, two pairs of lateral leaflet primordia. The primordium of the second foliage leaf shows a pronounced swelling just above its base, which indicates the point of origin of the acropetally developing lateral leaflet primordia; hairs are developing on the ventral surface of this primordium. The tiny primordium of the third foliage leaf lies just under one of the lower lateral leaflet primordia of the first foliage leaf. Compare this situation with the position of the youngest foliar primordia during scale formation (first foliage leaf: 286 μ × 429 μ; second foliage-leaf primordium: 214 μ × 321.75 μ. × 86).

FIG. 23. Ventral aspect of the seventh scale, lateral aspects of the first foliage leaf and the primordium of the second foliage leaf, April 30. This figure emphasizes the abrupt transition from the last scale to the first foliage leaf as well as the profound divergence between these organs at an early ontogenetic stage. In this bud, the first foliage leaf has formed three pairs of lateral leaflet primordia from which the numerous hairs have been removed to show details. The region below the lowest leaflet primordium will give rise to the petiole and the leaf base (cf. also FIG. 22). The primordium of the second foliage leaf is partially covered by the lowest lateral leaflet primordium of the first foliage leaf. (Seventh sc.: 858 μ long; first foliage leaf: 286 μ × 429 μ. × 52).

FIG. 24. Ventral aspect of the second foliage leaf, lateral aspect of the primordium of the third foliage leaf at the growing point, April 30. A developmental stage following that shown in FIG. 22. Note the prominent ventral hairs on the second foliage leaf which has formed two pairs of lateral leaflet primordia (second foliage leaf: 214.5 μ × 286 μ. × 118).

FIG. 25. Ventral-lateral aspects of the third foliage leaf and the primordium of the fourth foliage leaf, at the growing point, May 3. A developmental stage following that shown in FIG. 24. Note the characteristic decrease in size of the successive pairs of lateral leaflet primordia in the third foliage leaf which is rapidly forming dorsal and ventral hairs. The primordium of the fourth foliage leaf as yet shows no distinctive ontogenetic characteristics (third foliage leaf: 250.25 μ × 393.25 μ. × 90).



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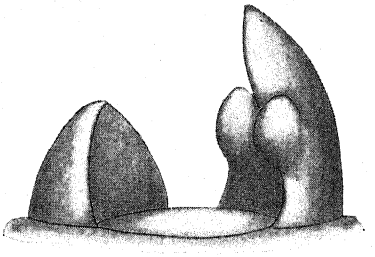

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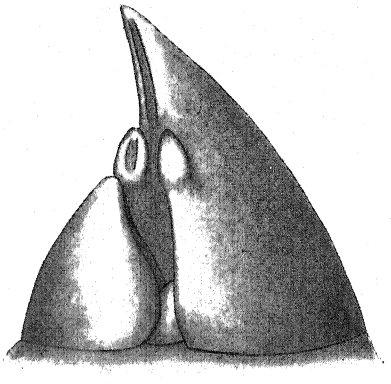

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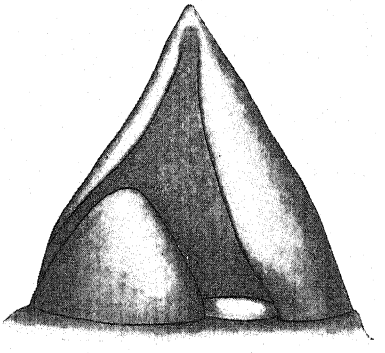

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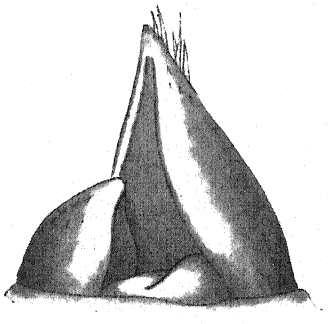

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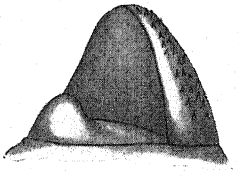

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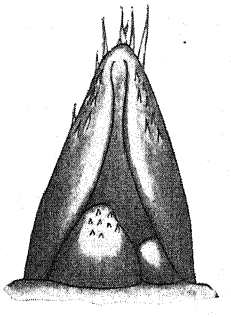

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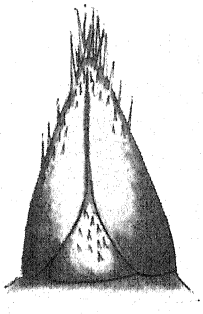

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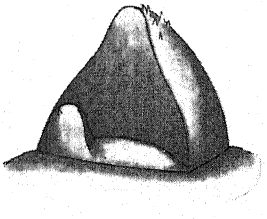

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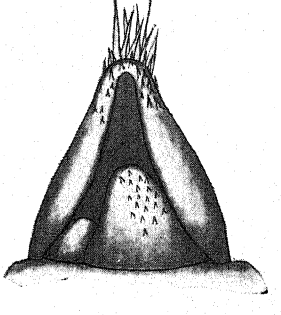

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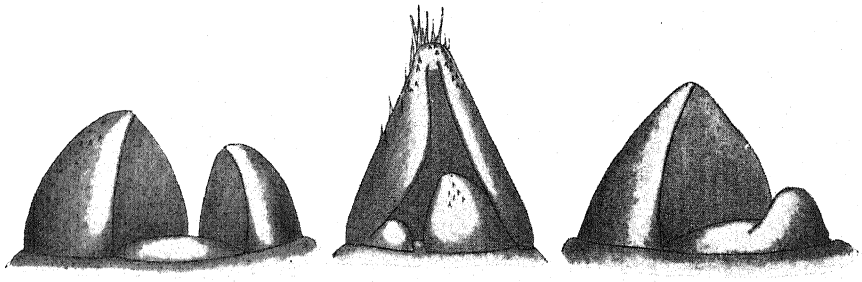
FOSTER: LEAF OF CARYA



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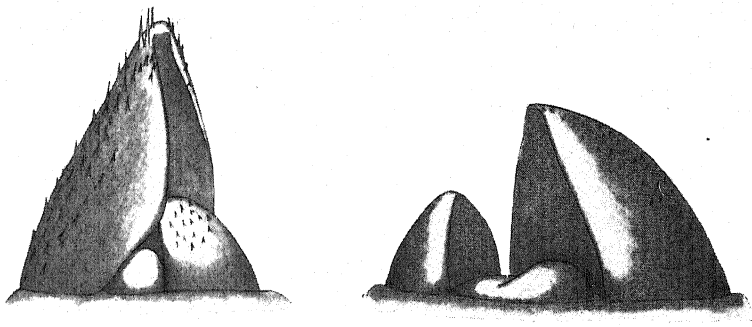

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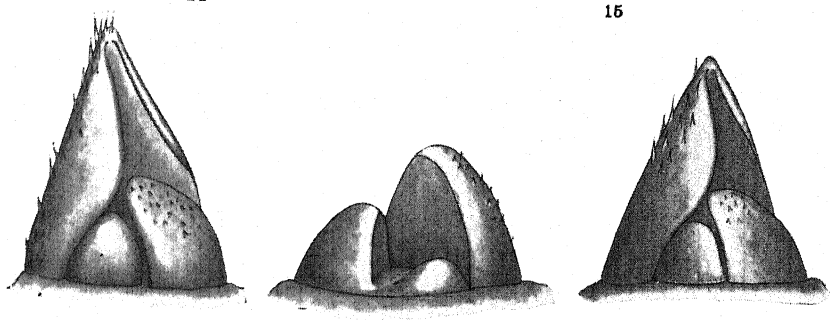

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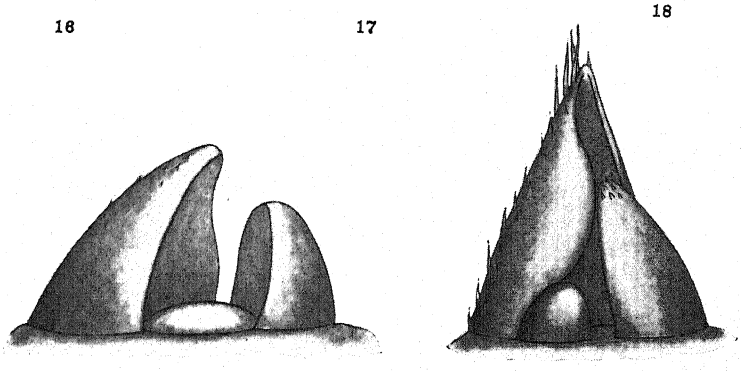

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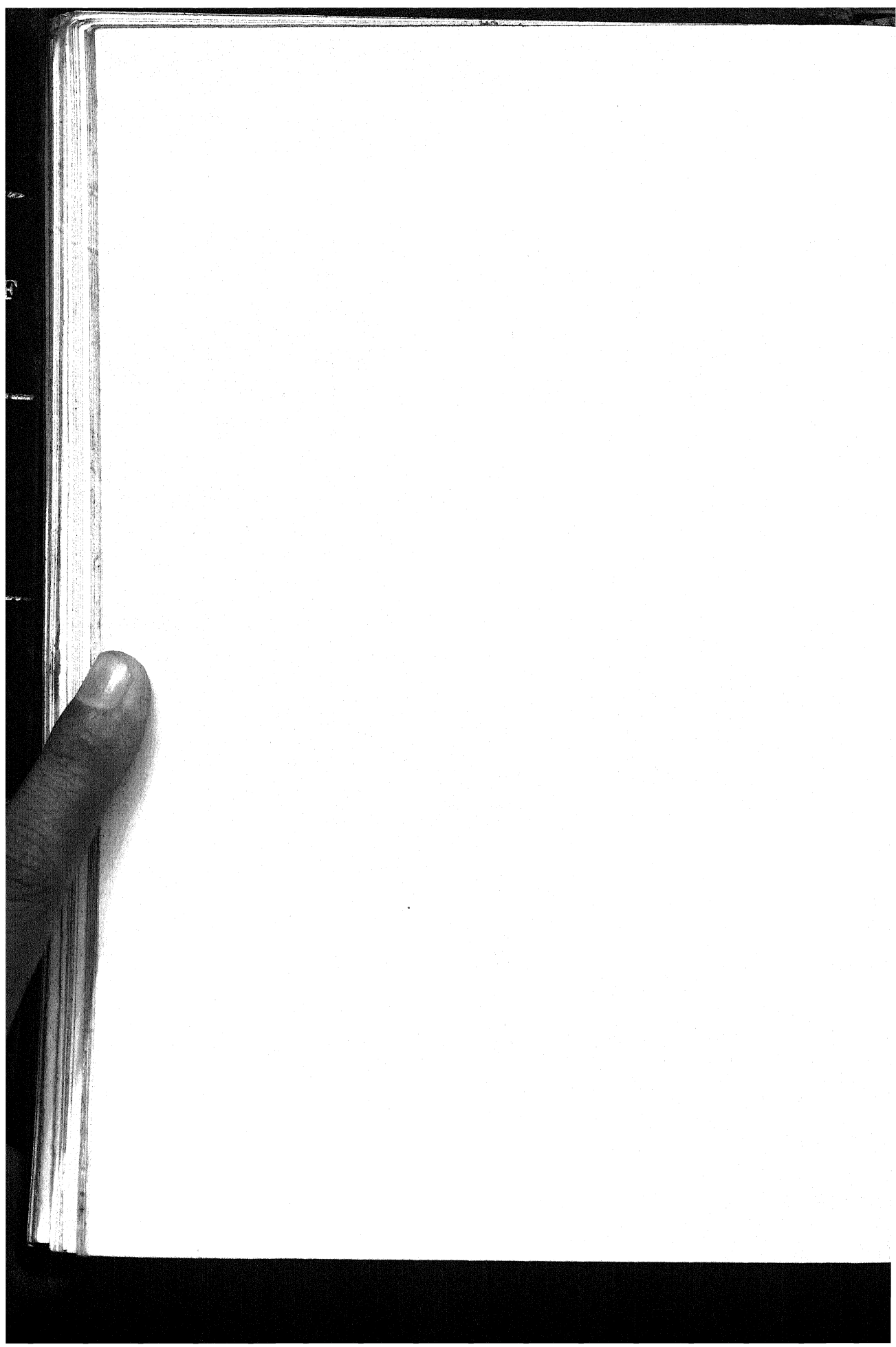

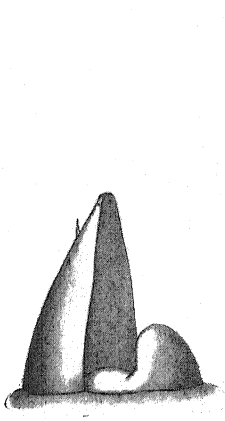

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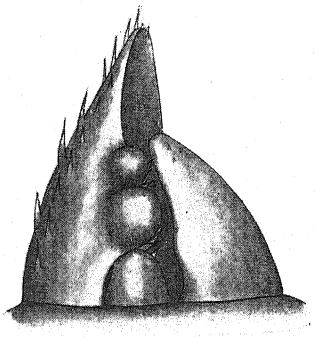

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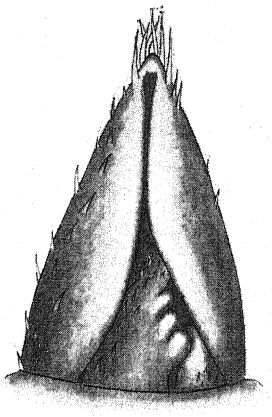

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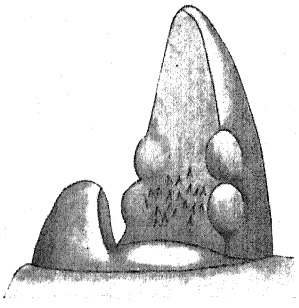

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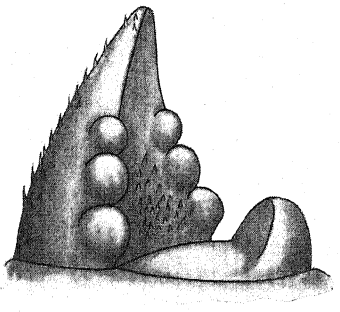

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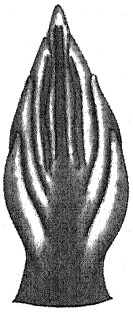

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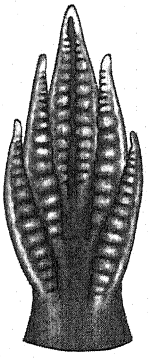

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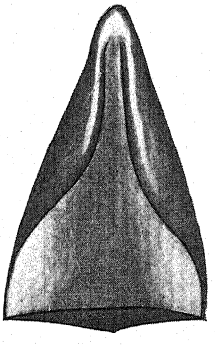

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FOSTER: LEAF OF CARYA

FIG. 26. Ventral aspect of the first foliage leaf dissected from a bud on May 8. All of the hairs have been omitted in order to show the finger-like form of the young leaflets and the initiation, in the uppermost leaflets, of blade development by the appearance of ventral meristematic weals. Note the prominent development of the basal region of the leaf at this stage in ontogeny (929 μ long. $\times$ 43).

FIG. 27. Ventral aspect of a foliage leaf dissected from a bud on May 13. A significant stage in development following that shown in FIG. 26; as before, all hairs have been omitted. Each leaflet now consists of a clearly demarcated midrib and a rapidly growing blade region. In the latter, closely-crowded, transverse ribs, corresponding to the main lateral veins of each leaflet, are appearing in acropetal succession. Notice the acuminate, rapidly elongating apices of the leaflets. The basal region of the leaf is growing slowly in basal width and thickness (1 mm. long. $\times$ 46).

FIG. 28. Ventral aspect of the fifth bud scale dissected from a bud on April 18; all hairs have been omitted for clarity. Note the prominent, asymmetrical wings which are growing most rapidly a short distance above the scale-base. This figure, when contrasted with FIGS. 26 and 27, emphasizes the utterly divergent mode of growth of the scale and the foliage leaf following the critical morphogenetic phases in the ontogeny of these organs (429 μ $\times$ 500.5 μ. $\times$ 88).

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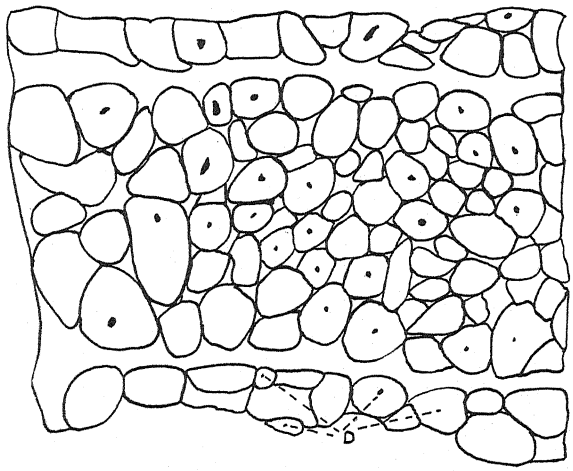
DEPOSITS OF OIL IN THE JUICE SACS OF CITRUS FRUITS[1]

WARD B. DAVIS

(Received for publication August 2, 1931)

In 1926–27, while examining individual juice sacs of Washington Navel oranges, the writer observed what appeared to be a globular body containing oil drops in the central part of each juice sac. Later Valencia oranges were found to contain a similar structure or body.

Tschirch and Oesterle (14) and Biermann (1) have studied the develop-



TEXT FIG. 1. Portion of a thin tangential section of a grapefruit. The deposits (*D*) are located in the centers of the cross sections of the juice sacs. (Drawn from a photograph.)

ment of juice sacs of citrus fruits, using the bitter orange for this purpose, but they do not mention any oil-bearing structure in the juice sacs. Other workers (2, 3, 4, 7, 8, 9, 10, 11, 16) dealing either directly or indirectly with the anatomy of citrus fruits do not appear to have seen such a structure in the juice sacs at any stage of development. Tunmann (15) and Molisch (6)

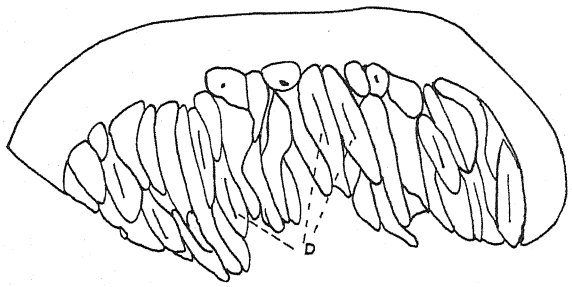
[1] Food Research Contribution No. 118.

[The Journal for January (19: 1–99) was issued February 3, 1932.]

discuss oil bodies, referring to them as elaioplasts, a term of doubtful significance (Sharp, 12). Neither these writers nor Haberlandt (5), who describes such deposits in detail, mention any deposit in the juice sacs of citrus fruits. That there is oil in the peel of citrus fruits is very well known, but its presence in the pulp appears to have escaped notice. The purpose of this paper is to direct attention to these oil deposits in the juice sacs, as well as to discuss their general nature and distribution.

In order to determine this distribution, sixty-nine varieties of citrus fruits were selected from the plantings of the Citrus Experiment Station of the University of California, at Riverside, California.

Macroscopically the deposits may be best seen by placing against a dark



TEXT FIG. 2. Longitudinal section of juice sacs of grapefruit. Deposits at *D*.

background a slice two to three mm. thick, made by cutting a cross or longitudinal section of the juice sacs as they occur in the locules. In the diagrams made from photographs (text figures 1 and 2) dark spots or streaks which appear more dense than the cellular matter surrounding them indicate the deposits in the centers of the cut juice sacs.

By the use of this method, as well as microscopical methods, all but seven of the varieties listed in Table 1 were found to contain the deposits. When available, immature and over-mature fruits as well as mature fruits were examined. In the varieties examined, if the deposits were present in the mature fruits they were also found in the immature and over-mature fruits. Eureka and Lisbon lemons contain the deposits in fruit at all stages of development, at least down to that in which the juice sacs are just large enough to be examined with the unaided eye.

The deposits of oil, wax, and granular matter are laid down in such a way that in many cells the shape of the entire mass ranges from an elongated type, as in grapefruit, to the more or less globular form found in some oranges. These masses vary in size and shape, and the oil, wax, and granular matter within them vary in amount. Granular matter is abundant in grapefruit, but in Bouquet de Fleur, in which wax is abundant, the granular matter is scarce. Relatively few of the juice sacs of the Dancy tangerine contain the deposits. The deposits are quite prominent in the trifoliate orange (*Poncirus trifoliata*), its hybrid, the citrange, and in grapefruit.

TABLE I. *Deposits of Oil in Juice Sacs of Citrus Fruits*

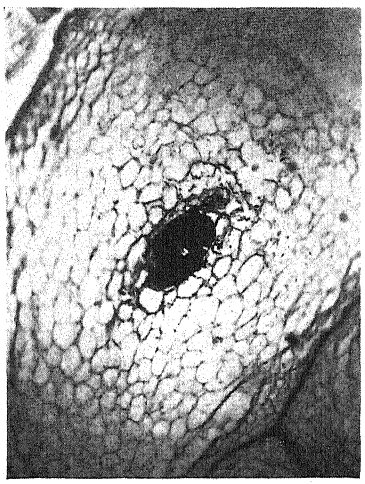
Variety	Deposit	Variety	Deposit
Citrus sinensis Osbeck		*Citrus Limonia* Osbeck	
Standard Valencia	+	Eureka lemon	+
Lue Gim Gong	+	Lisbon lemon	+
Parson Brown	+	Ponderosa lemon	+
Ruby Blood	+	Orange lemon	+
Seedling Sweet	+	Chinese lemon	+
Standard Washington Navel	+	Sweet lemon	+
Budsports of Washington Navel.		Rough lemon	−
Thomson Improved	+	Palestine sweet lemon	−
Golden Buckeye	+	*Citrus aurantifolia* (Christom.) Swingle	
Navelencia	+	Tahiti lime	+
Golden Nugget	+	Rangpur lime	+
Brown Spotted	+	Kusia lime	+
Australian	+	Bearse Seedless lime	+
Citrus Aurantium (L.), Bitter Orange		Sweet lime	+
Paraguay sour orange	+	Sweet lime	+
Bouquet de Fleur	+	Citrus limetta	+
Brazilian sour	+	*Citrus maxima* (Burm.) Merrill	
Sour orange variant	+	Round (Hawaii) shaddock	+
Sour orange variant	+	Duncan grapefruit	+
Sour orange variant	+	Frizzelle pink grapefruit	+
Sour orange variant	−	Foster pink grapefruit	+
Citrus nobilis Lour.		Marsh seedless grapefruit	+
King orange	+	Triumph pomelo	+
Willow-leaf mandarin	+	Hybrids and Miscellaneous Types	
Clementine mandarin	+	*Microcitrus virgata*	+
Citrus nobilis var. *deliciosa* Swingle.		*Poncirus trifoliata* Raf.	+
Weshart tangerine	+	*Fortunella margarita*	+
Trimbele tangerine	+	Yuzu Japanese orange	+
Dancy tangerine	+	*Poncirus trifoliata* x *Citrus sinensis*	+
Citrus nobilis var. *unshiu* Swingle		*Citrus mitis* x *Microcitrus australasica*	+
Owari satsuma	+	Morton citrange	+
Citrus Webberi var. *montana*	+	Savage citrange	+
Citrus mitis Blanco	+	Rusk citrange	+
Citrus hystrix DC.	+	Colman citrange	+
Citrus medica L.		Cunningham citrange	+
Citron of commerce	−	Sanford citrange	+
Italian citron	−	Limequat	+
Indian citron	−	Shaddock x St. Michael's orange	+
Tihi Tihi citron	−	Tangelo	+

They may also be observed without a lens in many varieties of oranges, such as Washington Navel, Valencia, and King. Although present in lemons and limes, they are relatively less abundant, smaller, and more likely to be overlooked. No citrons thus far examined contain them.

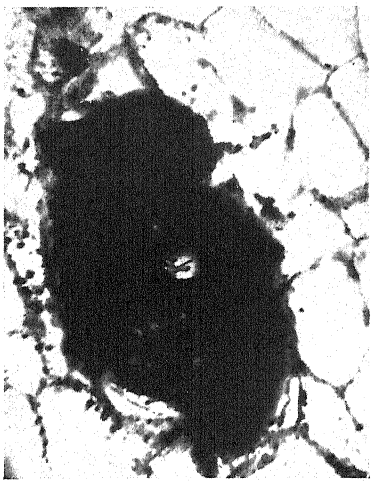
A study of the deposits in temporary mounts of the tissue was possible, because in some varieties the mass of cells containing the deposits was easily secured with little injury. After the ends of the juice sacs were cut off, the fruit was squeezed in such a way that elongated masses of cells containing the deposits were forced out of the juice sacs, and were thus available for observation in the juice or in water, in watch glasses with a dissecting microscope,

or on a slide with a compound microscope. In all varieties the glistening yellowish oil globules could be seen with a magnification of 10 to 16 diameters. In fact in a few varieties the oil globules were visible without magnification.

Under higher magnification, the cell walls in the vicinity of the deposits appeared thin. The addition of a cover glass to a mount on a slide often caused the delicate cell walls to tear apart, causing the collapse of the tissue. After the collapse the smaller oil globules flowed together, forming larger



TEXT FIG. 3. Cross section of juice sac of Eureka lemon, showing the oil deposit. × 50.



TEXT FIG. 4. The oil deposit of a section similar to that shown in text figure 3. × 2000.

ones, and the whole tissue quickly disintegrated, making it difficult to see the cell walls.

The relative size and arrangement of the oil globules may be seen to better advantage in histological sections as shown in text figures 3 and 4. The tissue was fixed in chromic-acetic-osmic acid solution [2] and the sections were cut after being embedded in paraffin. The osmic acid stained the oil black, and the cell walls were brought out by staining with light green. In many cases, as illustrated in text figure 4, the oil globules were large enough to fill several cells. The grouping of the oil globules along the cell walls is clear in this figure also. This arrangement sometimes makes the tissue containing the deposits appear fibrous under low magnification. It is of interest to note that the deposits occur entirely separate from fibrovascular tissue.

This study is preliminary to a chemical investigation of the deposits and their effect on the flavor and keeping quality of juices expressed from citrus fruits.

Thanks are due to Dr. H. J. Webber and other members of the staff of

[2] A method suggested by Dr. C. J. Chamberlain.

the Citrus Experiment Station, University of California, Riverside, California, for material and for aid in selecting citrus varieties from the plantings at the Station, and to the Botany Department of the University of Southern California at Los Angeles, for the use of histological apparatus.

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THE GAMETOPHYTES OF *SELAGINELLA KRAUSSIANA*
I. THE MICROGAMETOPHYTE

RODNEY A. SLAGG[1]

(Received for publication August 15, 1931)

The purpose of the present studies has been to obtain, if possible, a more reliable account than is at present available of gametophyte development in a single species of *Selaginella*, directing attention especially to the male gametophyte whose developmental story is less clear. *Selaginella Kraussiana* (Kze.) A.Br. was selected, since material was available readily in quantity, and since it is a species used widely as laboratory material in colleges and universities.

PREVIOUS WORK ON THE MICROGAMETOPHYTE

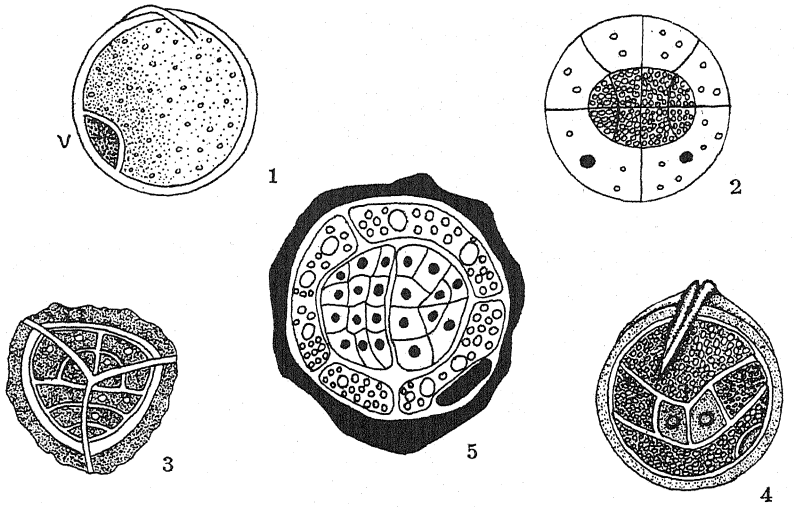
The first thorough study of the development of the microgametophyte in *Selaginella* was made by Millardet (14, *S. Kraussiana*). After breaking away the outer wall layers of the microspore, he observed through the transparent endospore the formation of a prothallial cell (text fig. 1) and of an eight-celled antheridium. Within the antheridium, in a manner unknown to him, arose two cells which formed a mass of spermatogenous cells. These then compressed and resorbed the jacket cells, and gave rise to antherozoids (text fig. 2). The prothallial cell remained intact.

Pfeffer (15, *S. Martensii*, *S. caulescens*) removed with acids the outer wall layers of the microspores, and through the endospore observed the formation of a prothallial cell and of an eight-celled antheridium (text fig. 3). He found, however, an orientation of walls wholly different from what Millardet had found, and he did not determine the succession of the walls. Moreover, except for the prothallial cell, all cells of the microgametophyte, including the jacket cells of the antheridium, served as primary spermatogenous cells, the primordial walls remaining intact. A very thin layer of slime, whose origin Pfeffer did not explain, collected eventually between the antherozoid mother cells and the old spore wall. Checking his revolutionary findings by examining some microgametophytes of *S. Kraussiana*, he was obliged to confirm Millardet's observations on that species.

Belajeff (1) examined ten species of *Selaginella*, and found them classifiable into two groups on the basis of microgametophyte development. Group I included *S. Kraussiana* and *S. Poulteri;* Group II, the remaining eight of the ten species which he examined. Microspores of Group I had spore walls of three layers; microgametophytes developed their antheridia

[1] The untimely death of Dr. Rodney A. Slagg occurred on August 9, 1931.

practically in the manner described by Millardet and confirmed by Pfeffer (text fig. 4), formed four inner (primary spermatogenous) cells, and one complex of antherozoid mother cells. Microspores of Group II had spore walls of only two layers; microgametophytes developed their antheridia in a manner somewhat similar to that of Group I, formed only two primary spermatogenous cells, but formed two complexes of antherozoid mother cells. In Group II the walls of the antheridial jacket were placed at angles different



TEXT FIGS. 1–5. 1–2 After Millardet. Redrawn from Sachs's "Lehrbuch der Botanik," 2nd ed. 1. Ripe microspore, freed of the exospore. At "*v*" the vegetative cell. 2. Cellular structure within the microspore; half-schematic; the single lines indicate the cell walls of the antheridium, the inner dark portion the cells from which issue the spermatozoid cells. 3. Redrawn after Pfeffer (15). Microspore showing the divisions of the endospore. 4. Redrawn after Belajeff (1). Germinating microspore in side view. From the cells of the second and third segments, the inner cells have been cut off. 5. Redrawn after Miss Lyon (13). Section showing the two cell complexes and their relation to the dissolving proteid masses by which they are enveloped. The masses of food matter are separated by strands of protoplasm.

from those of Group I. In both groups the jacket cells disintegrated eventually, and the antherozoid mother cells floated free in the slime thus produced.

Miss Lyon (13), for *S. apus*, gave an account of microgametophyte development widely different from the accounts of previous workers; and for "the American form" of *S. Kraussiana* she found "the sequence of events identical with those that obtain in *S. apus*." She questioned the certainty that "the species of *Selaginella* called *Kraussiana* in America is the identical one known by that name in Europe." In *S. apus* a wall-less prothallial cell was formed, then two cells were formed by free cell formation centrally in the old spore cavity. The protoplasm which enveloped the central cells sent out

"radiating processes which incompletely divided the spore cavity into irregular chambers, each of which was filled with granular masses of various shapes and sizes." The two central cells built up two uniform masses of antherozoid mother cells which at length floated in a slime produced by the disorganization of the food granules (text fig. 5). Jacket cells were not formed. Miss Lyon, having obtained her findings after the first use, by any worker on this problem, of modern technical methods, left it an open question as to how much the discrepancies were "due to differences in methods of technique, rather than to specific characteristics."

Campbell (6, 7), after re-investigating microgametophyte development in *S. Kraussiana*, was certain that "the masses surrounding the groups of sperm cells" were "cells, and not merely structureless masses of food-stuff developed in vacuoles" (6). He could not demonstrate nuclei in these cells, however (6), and made no attempt to verify the succession of the walls (7). He insisted that "the formation of the groups of sperm cells by a process of free cell formation" . . . was "improbable, and must be positively demonstrated before it can be accepted" (6).

Materials and Methods

The material for the present studies was obtained from the greenhouses of the Botany Department of the University of Wisconsin during the summers of 1929 and 1930. Specimens submitted to Dr. G. L. Grant of the Missouri Botanical Garden and to Mr. A. H. G. Alston of the British Museum (Natural History) were by both authorities identified as *S. Kraussiana*. Mr. Alston kindly furnished a complete synonymy of the species, giving the final determination as *S. Kraussiana* (Kze.) A.Br.

Strobili of as many stages of development as could be selected were picked and fixed in various fixatives. Also the entire sward was clipped and the plants laid to dry in covered trays lined with paper. Dehiscence of the sporangia threw out the spores which sifted to the paper, whence they could be gathered. Sowings of the spores were made on moistened Plaster of Paris blocks kept standing in distilled water in pans covered with glass and out of bright sunlight. After ten days, portions of the material were collected at one-day intervals and fixed in various fixatives.

Flemming's weaker and medium strength fixatives and formal-acetic-alcohol all gave satisfactory results; less so, Carnoy's fixative and Benda's fluid. Of the latter two, Carnoy's gave good fixation, but staining was unsatisfactory after its use; and Benda's did not seem to fix well. In attempts to find division figures, two collections of material were fixed, as Miss Lyon (13) suggested, in boiling hot fixatives, one of Flemming's weaker fluid, the other of formalin-acetic acid-corrosive sublimate.

Frequently considerable difficulty was experienced in getting satisfactory infiltration by paraffin. Even when infiltration seemed satisfactory, material in older stages of development proved difficult to section. Attempts

were made to aid infiltration and sectioning by treating fixed material with a one-half to one percent solution of hydrofluoric or hydrochloric acid. Either acid aided considerably, producing only slightly deleterious effects on the protoplasm. Good results also, from the standpoint of sectioning, were obtained after the use of a one-fourth to one-half percent strength of Jeffrey's fluid, and there was only slight plasmolytic or disintegrative effect; but even a brief exposure to weak solutions of Schultze's fluid was ruinous. An attempt was made to adapt Williamson's cellulose acetate method to the purpose, but while softening occurred and good sections could be cut, its action was too violent upon the protoplasm for it to be helpful. Softening and good sectioning could be accomplished also after the use of one-half per cent strengths of caustic soda or caustic potash.

When Pfeffer's method (15) of removing the exospore was tried, microspores were immersed for from twelve to sixteen hours on a glass slide in a drop of highly concentrated chromic acid, to which had been added "a little" sulfuric acid. While the exospore could be removed by this method, the action of the acids upon the protoplasm was so violent that the method seemed worthless. Finally the use of all softening agents was abandoned for the added reason that even prolonged washing, continued at times for more than three days, failed apparently to remove completely the chemicals, since normal staining reactions were not obtained.

The method of Millardet (14) and of Belajeff (1) was attempted; viz., that of rolling the mounted specimens beneath the cover glass until the exospore was loosened and removed, then treating the material with potassium iodid-iodine solution and making observations through the endospore. The writer found, as did Miss Lyon (13), that this was "a tedious performance whose results hardly repaid the effort."

Xylol proved undesirable as a paraffin solvent. It seemed to make the wall layers of the microspores more brittle and to cause the protoplasts to collapse. Even the precautions observed by Miss Lyon of passing the material from pure alcohol to pure xylol in seven steps, each taken after a twenty-four-hour interval, did not wholly prevent the defect. Chloroform was substituted.

A collapsed condition, traced to the time of introduction of the paraffin, was overcome by adding the paraffin of low melting point in finest shavings very gradually over a period of four or five days until the point of saturation at room temperature was reached. The material was then passed through paraffin of melting point 45° C. into paraffin of melting point 52° C., then of 58° C., in which it was allowed to stand for from two to three weeks. Prolonged soaking of the paraffin blocks in water, say for a period of three months or more, greatly facilitated sectioning. For cutting, there was used a knife of specially hardened steel, such as one ordinarily uses with a freezing microtome.

Difficulty was experienced in the making of ribbons, in that the sectioned

material tended to fall from place. Dr. H. Duerden of Birkbeck College, London, recommended in a personal letter a modification of Miss Lyon's procedure. The paraffin was removed from the oven at 58° C., and the temperature raised a little by warming briefly over a flame. Material was then poured quickly into the embedding dish and cooled rapidly in ice water. Microgametophytes were sectioned at four microns.

Chlor-calcium-iodid and chlor-zinc-iodid were used in tests for cellulose; Millon's reagent, in the test for protein. Flemming's combination of safranin, orange G, and crystal violet was used most frequently for staining, although some preparations were made with acid fuchsin alone, and some with a combination of methyl green and acid fuchsin counterstained with erythrosin.

Microspores

The close union of microspores in tetrads (Pl. V, fig. 9) frequently has not been disturbed even by dehiscence of the microsporangia. This habit of clinging together leads to the peculiar fashioning of the microspores. There is a doming of their free surfaces and a flattening of the surfaces of contact. Thus a ripe microspore freed from a tetrad (fig. 10) has somewhat the form of a tetrahedron; its base, however, is rounded.

In attempting to visualize the locations of cells or the direction of planes of cleavage in a developing microgametophyte, it is helpful to keep in mind the peculiar construction of the microspore. One may designate the trihedral angle as the apex of the microspore, the rounded surface as the base, and the plane surfaces as apical faces. Pfeffer (15) designated the three ridges diverging from the apex as apical ridges, and the less clearly-defined ridges formed where the three apical faces merge into the domed base as annular ridges. It is convenient to refer to the ill-defined points where apical and annular ridges meet as lateral coigns, and the axis passing from apex to base as the main axis of the microspore. The entire portion of the microspore above the level of the annular ridges will be designated the apical "half," and the portion below that level as the basal "half."

The base of the microspore is covered thickly with spines of varying lengths. Some are mere papillae; commonly there are others having a length equal to, or greater than, one-fourth of the diameter of the microspore. Some spines are straight and awl-shaped; others are crescentic; others, branched. From the three apical faces, spines are practically absent. Along each apical ridge runs a seam which may gape slightly, especially in sowed microspores (fig. 10); but in microspores still contained within the microsporangium, the seam is more apt to be puckered into a longitudinal crest (fig. 7), which in section appears like a nipple (fig. 17). The outer layer of the microspore wall thins at the apex to about half its thickness elsewhere (Pl. VI, fig. 19); and due, apparently, to crowding within the microsporangium, some microspores may be compressed into ovoid form (fig. 20). The plane of section of prepared mounts can usually be determined exactly when all these facts are considered.

Microsporangia

By the time the microsporangia have attained a diameter of 85 μ (Pl. V, fig. 1), one readily distinguishes in them three regions: wall, tapetum, and sporogenous tissue. The wall is uniformly two layers of cells in thickness, the outer cells columnar in shape; the inner, decidedly tabular. Tapetal cells also at maturity are columnar, the width of the tapetal layer equalling or surpassing that of the two layers of wall cells combined (fig. 2). Sporogenous cells are recognizable by their thicker, more darkly staining walls (fig. 1), and their more abundant, more deeply staining cytoplasm.

Denke (8, *S. Emiliana*) reported and figured for division of the tapetal cells that the orientation of the spindle would lead one to expect a transverse division of the cells, yet longitudinal division ensued. But in all of the mitotic figures encountered by the present writer in the tapetal cells of *S. Kraussiana* (text fig. 6), only transverse orientation of the spindle occurred. Bower reported concerning sporangia: "Abortive sporangia are to be found at the base of the strobilus" (3). In *S. Kraussiana* abortion of the apically placed microsporangia also is common. This abortion may be complete (text fig. 7), or only partial (text fig. 8). In the latter case some microspore mother cells produce apparently normal microspores.

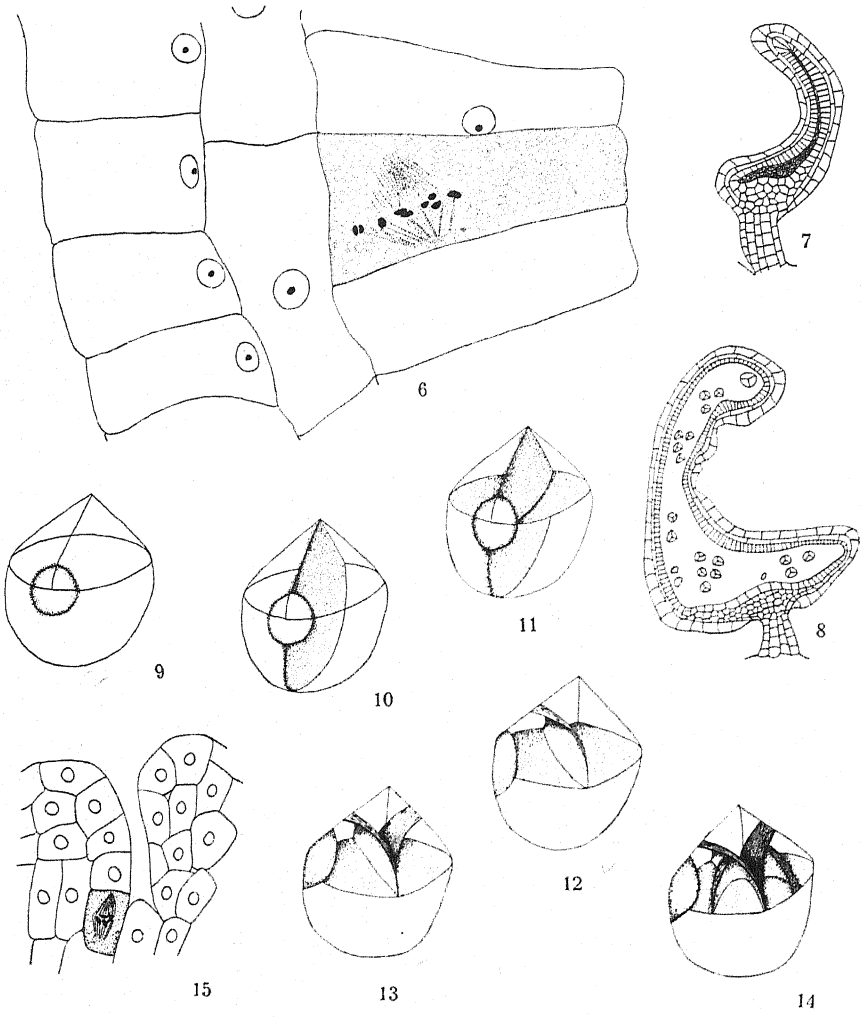
When the microspore mother cells float free in the fluid contents of the microsporangium (Pl. VI, fig. 2), they number about 175, and if each should form a tetrad of microspores, the total output per microsporangium would be about 700. It seems that this number is never attained, however. Many of the microspore mother cells fail to divide, and in some sections in which microsporogenesis seems completed, they may be seen still lying among the tetrads. Eventually such microspore mother cells disintegrate. The total output of microspores per microsporangium is about 600.

Not only does abortion occur in microsporangia and in many microspore mother cells, but also in many microspores. Frequently one, two, or three of the members of a tetrad fail to develop; or one finds whole tetrads, the separation of whose members was never completed (Pl. VII, fig. 32). In such cases the protoplasts are missing, and the tetrad is merely a group of empty shells.

Maturation of the Microspores

A microspore mother cell just previous to division (Pl. V, fig. 3) is almost isodiametric, and possesses abundant and finely reticulate cytoplasm in which a few granules occur. The nucleus contains a large, centrally placed, brightly staining nucleole, and a large amount of clear nuclear sap. Only meager strands of chromatin-linin are demonstrable. About each microspore mother cell appears a delicate, scarcely stainable wall.

No attempt was made to work out the cytological phenomena involved in microsporogenesis. Excellent synaptic figures (fig. 2) were encountered in the microspore mother cells, and occasionally a fairly typical division figure from one or the other reduction division. Commonly, however, karyokinesis



TEXT FIGS. 6–15. 6. Portion of wall layers and tapetum of microsporangium (×4500). Mitotic figure in tapetal cell. 7. Wholly aborted microsporangium (× 200). 8. Partially aborted microsporangium (× 200) containing a partial complement of apparently normal microspores. 9–14. Schematic. 9. Division of microspore into *prothallial* and *antheridial cells*. 10. Bisection of antheridial cell. The *first primordial wall* separates the *longitudinal halves*. 11. The *second primordial wall* arises near the level of the annular ridges. "Quadrant stage" of antheridium. 12. Longitudinal section of microgametophyte taken along plane of first primordial wall. Thus one-half of the *third primordial wall* is seen separating the proximal, smaller from the distal, larger cell. 13. *Fourth primordial wall* appears. 14. Periclinal walls arise, delimiting the two *primary spermatogenous cells* of each longitudinal half. Cf. FIG. 31. 15. Longitudinal section of bud tip, showing mitotic figure.

seemed irregular. Spindles were ill-formed; chromosomes lagged, and failed even to be included in the re-organized daughter nuclei; and portions of (apparently) chromatin material were rejected into the cytoplasm (fig. 5). No dependable chromosome count could be made, since chromosomes are very small, and some resembled so closely other granules in the cells that unmistakable identification of them was impossible.

The four microspores remain for a time enclosed as a group by the frail microspore mother cell wall (fig. 6). This becomes transformed, apparently largely by deposits from the tapetal fluid, into a common exospore about the tetrad (figs. 14, 32). By infolding of the exospore along the planes established during cell division, the microspores are finally parted. The triradiate apical ridges of each microspore become surmounted by seamed crests comprised of the united edges of the infolded, sheathing exospore (figs. 7, 8).

The exospore is at first smooth. When the microspore has enlarged to a diameter of about 20 μ, papillate projections appear upon the outer surface. These grow into long spines.

Sectioned material shows now a new wall layer, the endospore, within the exospore, the two layers not in close apposition (fig. 14). During growth of the microspore, the exospore outstrips in growth the endospore, and the gap between the two becomes widened. Apparently, too, the endospore may grow faster than its included protoplast, leaving the latter suspended as a vesicle at the apex of the microspore (fig. 14). Later the protoplast overtakes the endospore (fig. 17), but an imperfect union between the wall layers always persists (figs. 20, 26, 29), and explains the ease with which the exospore may be removed by rolling mounts beneath the cover glass.

A microspore at the time of completion of a tetrad (fig. 6) has a main axis of about 5 μ length. The arc of its base is somewhat more than twice as long. The microspore possesses scanty cytoplasm which is rather broadly reticulate; the vacuoles are turgid with a clear sap. The nucleus is relatively large ($D = 2\ \mu$) and spherical, and occupies an approximately basal position. A nucleole may be lacking, or one or more small nucleoles may be present. Chromatin-linin forms a scanty reticulum, more densely aggregated locally.

During the infolding of the exospore and the growth of the spines, the protoplast undergoes a series of changes. A large central vacuole appears in the cytoplasm, which is thus forced to a peripheral position (fig. 14). Here it appears in meager amount, frail, finely reticulate, and only slightly stainable, but possessing a few scattered granules which stain brightly with safranin. The nucleus has now migrated to an apical position, and becomes squeezed into an ovoid form by the turgor due to cell sap.

Gradually the cytoplasm increases in amount, decreasing the size of the central vacuole (fig. 16). The nucleus resumes its spherical form, and migrates along the wall (figs. 17, 18) to a basal position. The diameter of the microspore is now about 33 μ.

Chance sections (fig. 16) indicate that during the rapid growth of the

spore coats, the protoplasmic vesicle is not always left suspended in an apical position, but may cling to the base of the microspore. Fitting (9) reported the same position taken by the protoplast of the macrospore of *S. spinulosa*, where the basal position was the usual one, although any chance position might be taken.

Marked anabolism now occurs in the protoplast. The central vacuole becomes wholly obliterated by the cytoplasm, which becomes denser and more finely reticulate, and stains deeply (Pl. VI, fig. 19). Numerous small granules appear, and the contents of the vacuoles become extremely turbid. Application of Millon's reagent to the granules shows them to be proteinaceous. It is extremely difficult at this time to prepare a section cut at even three microns in such a way as to show sharpness of detail.

That the increased anabolism of this period has made heavy demands upon the reserve food of the microsporangium is indicated by the fact that the tapetal layer wholly disappears, except for an occasional nucleus. The marked anabolic phase seems to be one of relatively longer duration than any phase which has preceded, or which immediately will follow, for sections showing the above-described conditions are obtainable almost certainly from any strobilus, while other given stages are less apt to be found.

Eventually the cytoplasm becomes clearer. Clarification may be due in part to the aggregation of the great numbers of protein granules into the fewer, larger protein crystals which begin to appear, each surrounded by a film of cytoplasm. In optical section the crystals appear usually polygonal, but they are sometimes crescentic, or irregular. They stain brightly with safranin, as do also the large nucleoles and the inner wall layer.

Initiation of the Microgametophyte

The nucleus migrates now apexward along the wall of the microspore to a position near one lateral coign (fig. 15). Here the first nuclear division occurs. One daughter nucleus moves to the center of the old microspore (fig. 20), the other remaining as the nucleus of the *prothallial cell*. This cell becomes enclosed from the center of the old microspore by a thin, doming "wall."[2] The entire remaining contents of the old microspore constitute the *antheridial cell*, which has a diameter ten to twelve times that of the prothallial cell (Pl. VI, fig. 20; text fig. 9).

The antheridial cell becomes bisected, and a wall is formed which passes vertically through the main axis and intersects, or just fails to meet, the wall of the prothallial cell (figs. 21–24; text fig. 10).[3] The daughter cells formed by this division now divide, and curved walls are formed, convex downward, which cut the main axis at about the level of the annular ridges (Pl. VII, fig.

[2] The author is uncertain concerning the nature of this limiting structure, and of other "walls" to be mentioned hereafter. See "Discussion."

[3] Hereafter this wall will be designated the *first primordial wall*. The two halves of the microgametophyte which it separates will be designated the *longitudinal halves*.

25). These new walls may intersect the wall of the prothallial cell, or pass slightly above it. They coincide with each other near the middle of the first primordial wall, and together form one slightly curved wall, convex downward (text fig. 11).[4] A median vertical section of a microgametophyte, taken now at right angles to the first primordial wall, shows the old antheridial cell to be unequally "quartered" (fig. 26).

Of the four cells derived from the former antheridial cell, only the two occupying the top "half" continue to divide. The first division of these cells has not been encountered by the writer. The positions and intersections of the walls present after the second division, however (fig. 27), make clear the mode of the first division. There was formed in each of the two cells of the top "half" an arching wall concave to the prothallial cell. These walls extended from the old spore wall about midway between the prothallial cell and the apical coign,[5] and curved to meet the second primordial wall at about the latter's middle (text fig. 12). The new arching walls coincide with each other where they intersect the first primordial wall.[6] There are now present in each top "quadrant" of the microgametophyte two cells. One of these is smaller and proximal to the prothallial cell; the other is larger and distal from it, and arches over the smaller cell (text fig. 12).

Each larger cell in the top "half" now divides, and an arching wall is formed which extends from the spore wall midway between the apex and the level of the annular ridges, and curves to meet the third primordial wall slightly below its middle (fig. 27; text fig. 13). These new-formed arching walls also coincide with each other where they intersect the first primordial wall.[7] Disregarding the prothallial cell, there are now present in each longitudinal half of the microgametophyte four cells. One is a large basal cell; the other three are smaller, and with respect to each other are placed as if two were the daughter cells of the third which has the appearance of being a parent apical cell (fig. 27; text fig. 13). Including the prothallial cell, there are now present within the old spore wall nine cells.

The two most apically located cells of the microgametophyte usually undergo no further divisions. The nucleus of each invariably is found lying deep in the "trough" between the arching walls of the underlying cells (figs. 27, 30, 33). Each of these underlying cells now divides, and there are formed about the products periclinal walls, each curved in harmony with the old spore wall (figs. 28–29; text fig. 14). The new periclinal walls extend from the second primordial wall and curve upward, meeting, respectively, either the third or the fourth primordial wall; and each periclinal wall of one longitudinal half coincides perfectly with its counterpart in the other longitudinal half along their common intersection with the first primordial wall.

Disregarding the prothallial cell, each longitudinal half now contains six

[4] Hereafter this wall will be designated the *second primordial wall.*

[5] That is, this would seem to be its source if viewed in a section prepared like fig. 27.

[6] Together they will be referred to as the *third primordial wall.*

[7] Together they will be referred to as the *fourth primordial wall.*

cells: four are jacket cells, and these enclose the two rather spherical quadrate cells, which are *primary spermatogenous cells*. The longitudinal halves impress the observer as being mirrored counterparts of each other. An entire microgametophyte at this stage of development contains thirteen cells: four primary spermatogenous cells, eight jacket cells, and the prothallial cell (fig. 31). The prothallial cell has undergone no division since its formation.

At the thirteen-celled stage there comes a pause in the development of the microgametophyte. Like the "basal-nucleate" stage in the microspore, this thirteen-celled stage in the microgametophyte is one of stability and longer duration. An observer is almost certain to get from a sufficiently old strobilus sections which show the thirteen-celled stage of the microgametophyte; but it requires much tedious sectioning to make preparations showing the various stages between the "basal-nucleate" stage and the thirteen-celled stage.

Dehiscence of the microsporangia and dissemination of the microgametophytes occurs usually at a time when the majority of the microgametophytes in any one microsporangium have developed to the thirteen-celled stage. Owing to differences in the rate of development, some microgametophytes are shed at an earlier stage, and some may be retained in the microsporangia until development has progressed much farther.

Further development is concerned chiefly with changes in the central complex of *spermatogenous cells*. Each primary spermatogenous cell divides, and there are formed two four-celled tiers of spermatogenous cells (Pl. VIII, fig. 33). Divisions then occur rapidly and with no discovered regularity (figs. 34–36). The nuclei decrease in size for a time with each succeeding division. At first the complex has a rather ovoid form (fig. 34), but gradually it becomes spherical (fig. 37). Median longitudinal sections through a microgametophyte, made in the plane of the first primordial wall, show a cleavage through the central complex at right angles to the first primordial wall and parallel with the main axis (fig. 36). Transverse sections made at the level of the annular ridges show two cleavages meeting at approximately right angles (fig. 37). One cleavage lies in the plane of the first primordial wall. Obviously the products of all four primary spermatogenous cells maintain themselves for a time as genetic groups. But the groups are visible only temporarily; eventually the cleavages become indetectible, the entire spermatogenous complex appearing as a more or less spherical mass (fig. 38).

In the meantime the protein crystals in the jacket cells disappear (figs. 34–35), and large vacuoles filled with a clear fluid arise in the cytoplasm. A clear fluid zone appears about the central complex also, replacing the cytoplasm of the jacket cells, and giving the appearance that the latter are being digested by the spermatogenous cells (figs. 34–35). The fluid seeps in between the spermatogenous cells, first along the planes of cleavage just

described, parting into visible groups again the cells of the complex. Gradually the fluid seeps among all of the cells, so that finally the genetic groups are disorganized; still there is maintained a fairly close spherical association of all of the cells during continuance of their multiplication (fig. 38). The walls between the jacket cells seem more resistant to disintegration than does the cytoplasm, and they remain intact for a time. Vacuoles do not extend through the planes in which they lie. Nuclei are still more resistant than the walls, and even after all trace of the walls between jacket cells has disappeared, nuclei, apparently intact, still float in the slimy envelope around the central cells. At length the sphere of spermatogenous cells floats free, somewhat apexward within the old spore wall, in the slime which occupies practically the entire lumen. Only the collapsed prothallial cell and vestiges of the cytoplasm of the jacket cells remain.

The prothallial cell remarkably resists complete disintegration, yet commonly it is found in a collapsed condition, its once doming wall depressed like a bowl (figs. 36, 37). This condition is encountered sometimes even in very early stages of the microgametophyte (fig. 21). Usually, eventually, the prothallial cell disintegrates completely, no trace of it appearing when antherozoids are formed, In only a few instances were there found uncertain vestiges of the prothallial cell during the period of spermatogenesis.

The ultimate divisions of the cells of the central complex give rise to the *antherozoid mother cells*. These comprise the spherical complex at the time that it is dissociated. A count of the cells comprising a fully-developed sphere gives, therefore, a check on the number of antherozoids formed. This number was found to be 256. After the antherozoid mother cells float apart (fig. 39), spermatogenesis occurs (fig. 40). A count of the antherozoids as they lay thus dispersed, each coiled about its protoplasmic vesicle, again showed 256 per microgametophyte. Miss Lyon (13) found about 128 in *S. apus*, and Millardet (14) reported between 500 and 3000 in *S. Kraussiana*. The latter number seems to the present writer to be unbelievably large.

About two weeks after microgametophytes have been sowed on moistened Plaster of Paris blocks, the first fully developed antherozoids emerge. In about three weeks after sowing, all of the microgametophytes have ruptured the enclosing walls. The process of dehiscence and extrusion of the antherozoids may be aided by lightly pressing the mounts beneath the cover glass. Rupture of the spore coats occurs along the seams above the three apical ridges. The slime within the old spore walls is expelled either in one mass or in little puffs, and the antherozoids are carried out. Usually they are still coiled about a vesicle of cytoplasm. This they soon cast off and swim away rapidly. The body rotates, and movements are random dartings. Material put aside and re-examined from time to time showed that after twenty minutes to half an hour activity ceased.

Discussion

The abundant inclusions in the cytoplasm, particularly of the jacket cells, makes difficult at times the interpretation of sectioned material. The protein crystals especially stain strongly, and are apt to obscure the nuclei. It was not until sections were made at three and four microns that the true cellular nature of the jacket was determined.

Protein crystals were never present in the central cells in their later stages, but occasionally a few crystals were encountered in the primary spermatogenous cells (fig. 28). In the jacket cells crystals were for a time large and abundant, but as development proceeded they diminished in size and number. There has been nearly unanimous agreement that they constitute a food reserve. The first small granules seen by the writer occurred in the cytoplasm of the microspore mother cells. Denke (8) thought that the granules at this time represented materials to be contributed to spindle formation, since they disappeared upon initiation of the kinoplasmic threads and reappeared in the daughter cells. He never, however, detected an actual extension or aggregation of these granules into threads. It seems rather that even at this time the granules may represent a food reserve, broken down during mitosis, and replaced by anabolism later. They stain like the more abundant and larger protein crystals which occur in the microgametophyte.

Since the jacket cells of the antheridium undergo a disintegration which keeps pace with the multiplication of the spermatogenous cells (figs. 34–38). it seems clear that it is they which contribute the slime in which later the antherozoid mother cells and antherozoids float. This was the view of Millardet (14) also, and of Belajeff (1). While Pfeffer (15) observed that a thin layer of slime collected between the antherozoids and the old spore wall, he did not explain its origin. The jacket cells of the antheridium he interpreted as sharing in the production of antherozoids. Their walls remained intact distinct among the spermatogenous cells. Undoubtedly (as Belajeff has pointed out) Pfeffer observed the microgametophytes too late in their development. Two weeks after sowing, the divisions of the inner (spermatogenous) cells easily might have been mistaken for divisions of the cells of the antheridial jacket. The thin film of slime may have been really the remains of the jacket cells which Pfeffer failed to see. Also Pfeffer's treatment of the old spore coats with chromic and sulfuric acids may have had a bad effect (Belajeff, 1); but Belajeff's assertion that Pfeffer "die Sporen blos von einer Seite beobachtet hat" (1) seems unfair, for Pfeffer described and figured microgametophytes in many views. Moreover, employing identical methods of observation upon microspores of *S. Kraussiana*, Pfeffer confirmed Millardet's account (14) of microgametophyte development in that species, and with this account, in its main outline, Belajeff and the present writer agree. Since Belajeff and Pfeffer differ so widely regarding microgametophyte development in *S. Martensii* and *S. caulescens*, it must be left to future

researches, employing modern technical methods, to determine the true developmental story in these two species. Miss Lyon (13) believed that in *S. apus* the slime within the old microspore wall arose by a disorganization of food granules in the vacuolate cytoplasmic envelope around the central spermatogenous complex. In *S. Kraussiana* she found an identical sequence of events. But the certain demonstration of the existence of a cellular jacket in *S. Kraussiana* makes now imperative a re-examination of the microgametophytes of *S. apus*.

Millardet (14, *S. Kraussiana*), Belajeff (1, *S. Kraussiana*), and Pfeffer (15, *S. Martensii, S. caulescens*) state that the prothallial cell, with cellulose wall, persists until the discharge of the antherozoids. Miss Lyon (13, *S. apus*) found no wall at all around the prothallial cell. The present writer finds a frail "wall," but in it no evidence of cellulose. This may explain its shorter duration than others reported.

Bruchmann (5) prefers to designate as *rhizoidal cell* what others have denominated *prothallial cell*. He would homologize it with the rhizoidal humps of *Selaginella* macrogametophytes, and with the rhizoidal cell formed by germinating fern spores. While it is true that the first cell formed by a germinating fern spore is located within the spore wall in a position fairly homologous to that occupied by the first cell formed in a germinating *Selaginella* microspore, the position alone hardly warrants homologizing the two cells morphologically. In the shape of the antherozoid of *Selaginella*, as described by Roze (16) and Belajeff (1), there would seem to be an indication that the affinities of *Selaginella*, as revealed by its gametophyte generation, are with the bryophytes and not with the ferns. Bower (3) has shown that the sporophyte, also, of the lycopods suggests bryophytic affinities, and Jeffrey (12) has shown that the Lycopsida differ fundamentally in their anatomy from the Pteropsida. It would seem more valid to homologize morphologically, on the basis of homologous position, the first-formed cell of the microgametophyte with the rhizoidal humps of the macrogametophyte. In *Selaginella* species one deals then with the germination products of two spores derived perhaps from an identical plant. If, as may be, heterospory has evolved from an ancestral homosporous condition (3), then one might expect to see emerge, during development of each type of spore, some common structure. It may be emphasized that in the macrogametophyte of *Selaginella*, it is uniquely a hump of *prothallial cells* which arises at each lateral coign (Bruchmann, 4), and from these prothallial cells the rhizoids arise later as specialized structures. In some species (e.g., *S. Kraussiana*) rhizoidal humps are formed only rudimentarily; some humps may bear no rhizoids at all. It seems clear that in the process of evolution, it is the prothallium which would be retained as a vestigial organ, and not the rhizoids, and there is little warrant for abandoning Millardet's early designation as *prothallial cell* the first meniscoid cell of the microgametophyte.

Furthermore, Bruchmann (5), reviewing the work of Belajeff (1), refers

to the *four antheridia* per microgametophyte in *S. Kraussiana* and *S. Poulteri*, and to the *two antheridia* of other species. He mentions all species as agreeing in the formation of *a few sterile prothallium cells*. Obviously Bruchmann regards as antheridia the primary spermatogenous cells, and as prothallium cells the cells of the antheridial jacket. Belajeff (1), Millardet (14), Pfeffer (15), and Campbell (6, 7) interpreted but a single antheridium per microgametophyte, for which the sterile cells, excepting the prothallial cell, comprised a multicellular jacket. Miss Lyon (13) found no antheridium in *S. apus*, only a *generative cell* in addition to the prothallial cell. It is true that Belajeff and Pfeffer use the term antheridium in two senses: first, as naming the sister cell (antheridial cell of the present paper) to the prothallial cell; later, as naming the completed structure comprised of fertile cells and sterile jacket. But no observer has described for one microgametophyte the formation of more than one antheridium. There seems to the writer no good reason to regard either the primary spermatogenous cells or the spermatogenous complexes as separate antheridia. Rather all are formed in one antheridium, in developing which the cells of the longitudinal halves arise in close synchrony. Up to the completion of the primary spermatogenous cells, homologous cell walls in the longitudinal halves coincide perfectly along their junctions with the first primordial wall; they form thus one completed unit comprised of eight jacket cells enclosing four inner, fertile cells. The complete microgametophyte of *S. Kraussiana* is properly conceived as comprised of a *prothallial cell* and a *single antheridium*.

In the walls of the jacket cells, Millardet (14) and Belajeff (1) were unable to demonstrate cellulose. Miss Lyons (13) found no jacket cells at all—only radiating strands of cytoplasm. Clearly, however, in *S. Kraussiana* one has to deal with complete cells separated by some sort of frail partition. The writer was unable to demonstrate in these "walls" a trace of cellulose, or to throw much light on their origin and nature by studying the phenomena of cell formation. Dividing nuclei were never encountered. Figure 23, however, is easily interpreted as displaying cell plate formation. If this interpretation be correct, then the beginnings of the cell plate shown in this figure resemble so closely the frail "walls" figured elsewhere that they might be regarded as an identical type of structure. Moreover, a chance section revealed the condition depicted in figure 24, which might be interpreted as the tearing of a cell plate along the plane by which, if development had continued, it would have parted naturally to form the plasma membranes of two daughter cells. These "walls" stain like the cytoplasm, and in thick sections are easily confused with it. Thus again they seem to resemble plasma membranes or the material of the cell plate. And certainly these "walls" are plastic like cell plates. A comparison of figure 25 with figure 28 shows this condition clearly with respect to the second primordial wall. A comparison of figure 27 with figure 28 shows the same condition with respect to the last "walls" laid down in the jacket. Hence the writer believes that the frail

partitions between the jacket cells are cell plates which never have cleaved into plasma membranes, and the cells comprising the antheridial jacket are, therefore, primordial cells which do not form true walls.

At the time of the disharmonies in growth of spore wall layers and protoplast described above, the presence of the large central vacuole made difficult the manipulation of material. But careful examination of material which showed good fixation convinced the writer that the disharmonies in growth are real; they are not plasmolytic effects. There is, therefore, essential agreement between microspores and macrospores with respect to this phenomenon of development.

Attempts by the writer failed to demonstrate nuclear division figures in developing microgametophytes, yet abundant evidence was found of the aberrant meioses described and figured by Miss Graustein (10). Heinsen (11) could demonstrate no mitotic figures in the macrogametophyte; but finding instances of elongate and of constricted nuclei, and of nuclei lying in close apposition, he believed that nuclear division in the macrogametophyte was by constriction. Examining bud tips from the sporophyte, he here, too, found biscuit-shaped or apposed nuclei. In the tips of strobili (text fig. 15) and in the tapetum (text fig. 6), the present writer found division figures in metaphase and anaphase. Miss Lyon (13) depicts mitotic figures in both of the gametophyte generations and in the sporophyte. There seems little doubt, therefore, that nuclear division by mitosis prevails in *Selaginella*.

Miss Lyon attributed to a thorough exposure of her material to boiling hot fixatives her success in finding division figures. She believed that these hot fixatives penetrated quickly and completely, and prevented a lingering death of the protoplasts, with accompanying loss of mitotic figures. The writer's use of this method, limiting, however, to from three to five minutes the exposure to the hot fixatives, was unsuccessful.

The "basal-nucleate" stage in the development of the microspore was described as one of increased anabolism and comparative stability. It seems to be the final phase in the maturation of the microspore, and immediately precedes germination into the microgametophyte. The first cell division seemed always to accompany clarification of the cytoplasm after this stage, and the commencement of the formation of the protein crystals.

In determining the succession of events in a developing microgametophyte, one gets only imperfect aid through comparing events in basal, with those in more apical, microsporangia. By no means do the microgametophytes develop with equal rapidity. Instances are encountered in which microspores in a "basal-nucleate" stage are present in the same microsporangium with thirteen-celled microgametophytes. Microspores with meager, peripheral cytoplasm and an apical nucleus occur sometimes in microsporangia in which most of the microspores have a basal nucleus, and turbid, granular cytoplasm. The rather fleeting stages in which the first to fourth primordial walls are laid down should be sought, therefore, not only in

microsporangia toward the tip of the strobilus, but in more basal microsporangia as well.

The thirteen-celled stage represents the final stage attained usually by a microgametophyte within a microsporangium. Occasional disseminated microgametophytes showing a less number of cells seem explicable as the laggards in development above described. While Millardet (14) reported microgametophytes shed sometimes at a five- or seven-, but usually at a nine-celled stage, he had failed probably to see at this time the four primary spermatogenous cells. He did observe at a somewhat later time two of them, but found it impossible "de rémonter plus loin dans l'histoire de ces cellules et d'assister à leur formation." Belajeff (1) found thirteen cells in a "ripe" microgametophyte of *S. Kraussiana*. Pfeffer (15) found nine to be the usual number in *S. Martensii* and *S. caulescens*, although seven was the usual number in one example of the last-named species. Miss Lyon (13) states merely that the latest stages of development occur in the strobili after they are shed.

During the latter part of August, 1929, the writer made a few fixations of late-appearing strobili, none of whose microsporangia had dehisced. The microgametophytes within the lower four or five microsporangia of these strobili had continued their development up to complete maturation of the antherozoids. Figures 39 and 40 depict sections of such microgametophytes obtained by cutting embedded strobili.

Retention of the microgametophytes within the microsporangia throughout development would seem to be an advantage, since the microgametophytes would be kept then close to a food supply. There is some evidence that an increased use of the materials of the microsporangium for food may be made by such microgametophytes, for the inner, tabular layer of wall cells in the microsporangium disintegrates, like the tapetum, and becomes part of the tapetal fluid. The nuclei of the wall cells may be seen, in fortunate sections, floating in the slime near the remaining wall layer. Microsporangia which shed their microgametophytes at the thirteen-celled stage retain their inner wall layer intact.

Miss Lyon (13) mentioned the development within both micro- and macrosporangia of a sub-archesporial pad, surmounting the pedicel. This pad, being in close relation to the vascular strand, she described as "probably facilitating," in macrosporangia, "the supply of nutriment to the sporangium wall until the embryos begin to form." It seems significant that the microsporangia also which retain their microgametophytes until development is complete show some proliferation of the sub-archesporial pad.

Concerning the entire developmental story of the microgametophyte of *S. Kraussiana*, the present writer agrees in the main with the findings of Belajeff (1) for those species which he placed in Group I. The succession and orientation of the walls have been found to be exactly as he described. The text of Millardet's monograph (14) gives a developmental story also es-

sentially like that of Belajeff, but his figures, which appeared in the second edition of Sachs's Botany (17), do not agree with his verbal account. Pfeffer (15) confirmed Millardet's findings for *S. Kraussiana*. Campbell (6, 7) corroborates Belajeff quite fully.

One can only speculate as to the reason for the very different findings of Miss Lyon (13). Is it possible that her "American form" of *S. Kraussiana* was incorrectly identified? She quotes no taxonomic authority. Moreover, the present writer found, at the beginning of his researches, that he could obtain, at seven and eight microns, sectioned material which looked much like some of Miss Lyon's figures. Walls and jacket cells could not be demonstrated. Miss Lyon does not state at what thickness her sections were cut. Is there the possibility that they were cut too thick to distinguish sharply the frail walls from the cytoplasm? The writer had no difficulty in demonstrating the walls when sections were cut at four microns.

The further question may be raised as to whether it is possible that the use of boiling hot fixatives, which proved of such service in fixing the nuclear division figures, had a disastrously disintegrative effect upon the jacket cells. These cells are naturally ephemeral. The primary spermatogenous cells display from the start an abundance of cytoplasm which contrasts sharply with that of the jacket cells. In the jacket cells there is so much fat and crystalline protein that it seems possible that treatment of these cells for a long time with hot fluids may have destroyed them and left only radiating strands of protoplasm. During the natural disintegration of the jacket cells which just precedes spermatogenesis, their thin "walls" seem more resistant than the cytoplasm to disintegration by the spermatogenous cells. Now if, during treatment with hot fluids, portions of the cytoplasm might have broken down and vacuolization occurred, the vacuoles would have become organized probably likewise *within* the frail "walls," they would not have extended *through* them. This would correspond to the condition which arises during natural disintegration of the jacket. Remaining shreds of cytoplasm would then cling along these walls and along the borders of the spermatogenous complex, and produce the effect of the latter being "free cells formed centrally, the protoplasm enveloping them sending out radiating processes that incompletely divide the spore cavity into chambers."

Heinsen (11), in his attempts to find nuclear division figures, subjected some of his macrogametophytes to one-and-one-half- to five-minute dips in boiling water, apparently without bad effects on the protoplasm. His failure to find division figures Miss Lyon ascribed to too brief exposure to the fluid. Future researches may show whether, by prolonged exposure, one obtains a disintegrative effect, whereas by briefer exposure (and certainly by the use of cold fixatives), although losing the division figures, one preserves the jacket cells.

Miss Lyon's figure 74 (13) is interesting in that it shows a fine black line extending like a radius through the protoplast of a microgametophyte; but

concerning this line she gives no explanation. It has the same general appearance as is possessed by the frail "walls" of the jacket cells in the microgametophytes of *S. Kraussiana* as shown by the present writer's researches. May it be that the line represents really a portion of such a "wall" which has survived exposure to the boiling fixative, and that a re-examination of *S. apus* may show a "sequence of events identical with those that obtain" in *S. Kraussiana?*

Summary

1. The total output of microspores per microsporangium is about 600.

2. The microspore nucleus, basally located in the microspore at the completion of microsporogenesis, moves to an apical position during organization of the large central vacuole. During the later obliteration of the vacuole, the nucleus migrates to a basal position again.

3. The *prothallial cell* is formed near one lateral coign. In its wall no cellulose could be demonstrated.

4. The *antheridial cell* becomes bisected vertically and the wall formed may interesect, or just fail to meet, the wall of the prothallial cell.

5. Each longitudinal half becomes divided by a wall, convex downward, near the level of the annular ridges. These two walls coincide along their intersection with the first primordial wall, and together constitute the second primordial wall.

6. In each upper "quadrant" two successive cell divisions produce three cells. These with respect to each other are placed as if two were the daughter cells of the third, which appears like a parent apical cell.

7. From the "daughter cells" arise four primary spermatogenous cells, bounded exteriorly by periclinal walls curved in harmony with the microspore wall.

8. Microgametophytes when shed are composed usually of thirteen cells: one is the prothallial cell; eight are jacket cells, and these enclose four primary spermatogenous cells.

9. Jacket cells disintegrate during multiplication of the cells in the spermatogenous complex.

10. The spermatogenous complex is composed temporarily of four genetic groups of cells, descendants respectively of the four primary spermatogenous cells.

11. A single microgametophyte produces 256 antherozoids.

The writer is indebted to Prof. George S. Bryan for advice and encouragement during the course of this study, which was undertaken at his suggestion and done under his direction in the Department of Botany of the University of Wisconsin. The suggestions of others regarding the preparation of material are appreciated, and thanks are extended to Dr. Grant and Mr. Alston who identified the species studied.

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EXPLANATION OF PLATES

Magnification is × 2120 unless otherwise specified.

PLATE V

FIG. 1. Young microsporangium, showing differentiation into wall layers, tapetum, and sporogenous tissue. × 760.

FIG. 2. Mature microsporangium. Microspore mother cells float free in tapetal fluid. × 760.

FIG. 3. Microspore mother cell just previous to meiosis. × 4500.

FIG. 4. Telophase of first reduction division. × 4500.

FIG. 5. Sextuple spindle of second reduction division. Aberrant behavior of chromatin material. × 4500.

FIG. 6. Newly completed tetrad. Nuclei basal, cytoplasm broadly reticulate. Wall of microspore mother cell forms common exospore. × 4500.

FIG. 7. Microspore as it usually appears just after dissociation of a tetrad. Exospore folded into crests over apical ridges.

FIG. 8. Chance preparation showing apical clefts already formed at time of dissociation of tetrad.

FIG. 9. Completed tetrad, the members still associated after dehiscence of microsporangium. × 1210.

FIG. 10. Completed microspore, apical view. × 1210.

FIG. 11. Completed microspore, basal view. × 1210.

FIG. 12. Empty "shell" (old microspore walls) of microgametophyte after escape of antherozoids. × 1210.

FIG. 13. Chance preparation showing microspore in apical view. Exospore (partly broken away) has grown away from endospore, and endospore from protoplast, leaving latter suspended apically. Nucleus in apical position.

FIG. 14. Longitudinal section through three members of a tetrad, showing disharmony in growth of wall layers and protoplast. Protoplasts, suspended apically, show meager cytoplasm, small protein grains, and large central vacuole.

FIG. 15. (Follows FIG. 19 in developmental story.) Cytoplasm clarified somewhat. Protein crystals develop. Nucleus has migrated apexward to a position near one lateral coign.

PLATE VI

FIG. 16. Chance preparation showing protoplast attached basally to spore wall. Nuclei still apical. Cytoplasm increases in amount.

FIG. 17. Longitudinal section through single microspore. Cytoplasm increases. Nucleus begins to migrate baseward.

FIG. 18. Nucleus in basal position. Cytoplasm pervades vacuole.

FIG. 19. "Basal-nucleate" stage. Central vacuole obliterated. Much oil and granular protein present.

FIG. 20. Prothallial cell formed near one lateral coign. Second daughter nucleus moves to more central position in antheridial cell.

FIG. 21. Transverse section through microgametophyte at level of annular ridges. First primordial wall bisects old antheridial cell vertically along main axis.

FIG. 22. The same as FIG. 21, longitudinal section.

PLATE VII

FIG. 23. The same as Fig. 22, beginnings of cell plate formation.

FIG. 24. The same as FIG. 22, cell plate torn, shows two plasma membranes forming.

FIG. 25. Longitudinal section in plane of first primordial wall, shows second primordial wall crossing microgametophyte transversely near level of annular ridges.

FIG. 26. Longitudinal section at right angles to plane of first primordial wall shows microgametophyte unequally "quartered" by first and second primordial walls.

FIG. 27. Longitudinal section in plane of first primordial wall. Former upper "quadrant" of one longitudinal half now divided into three cells. Third primordial wall concave to prothallial cell. Fourth primordial wall intersects the third just below the latter's middle.

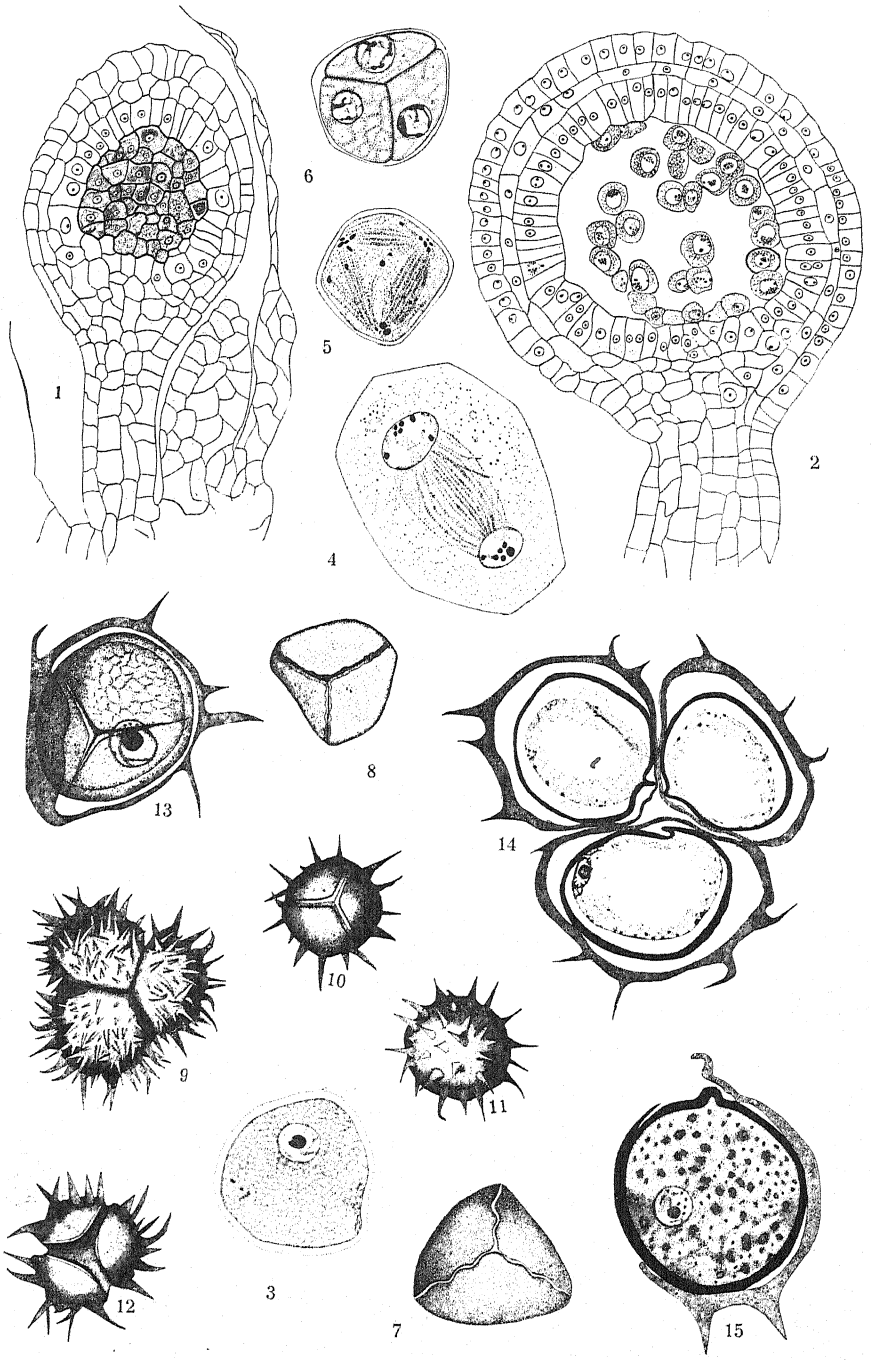
FIG. 28. Longitudinal section in plane of first primordial wall. Periclinal walls separate the jacket cells from the two primary spermatogenous cells of one longitudinal half. Instance in which second primordial wall failed to meet prothallial cell.

FIG. 29. Transverse section taken just above level of annular ridges at same stage depicted in Fig. 28. Four primary spermatogenous cells in completed antheridium.

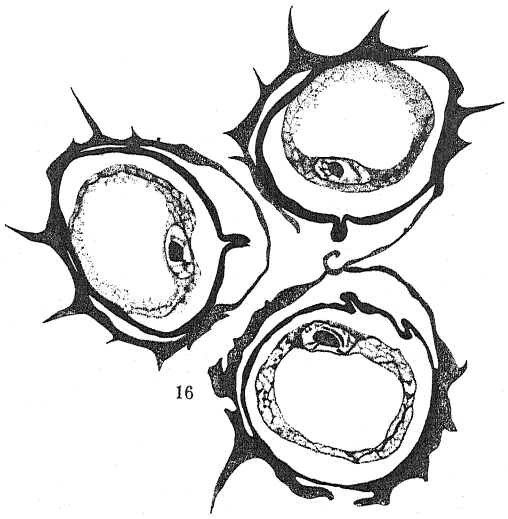
FIG. 30. Longitudinal section parallel with plane of first primordial wall. Shows unusual instance in which basal cell of antheridium underwent re-division. Prothallial cell (not shown) occurred in next section of series.

FIG. 31. Schematic representation of microgametophyte in thirteen-celled stage. Section, in plane of first primordial wall, shows mirrored longitudinal halves.

FIG. 32. Aborted tetrad; dissociation never completed.



SLAGG: GAMETOPHYTE OF SELAGINELLA



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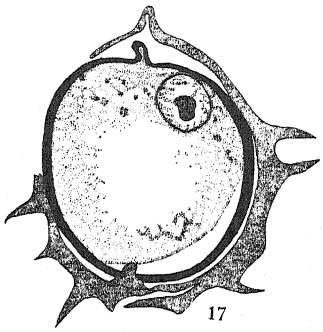

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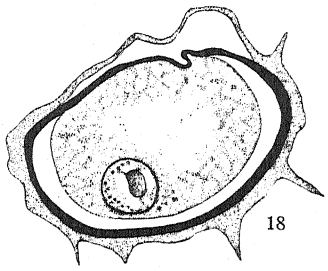

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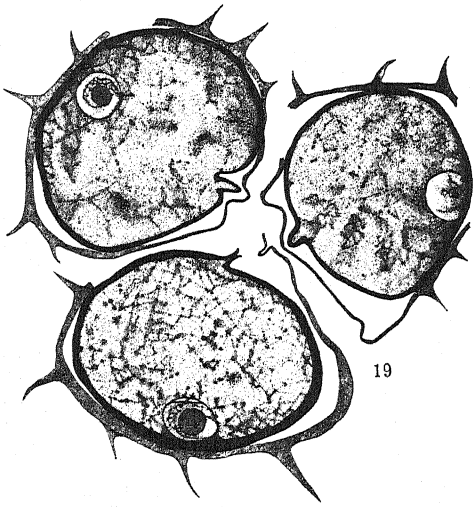

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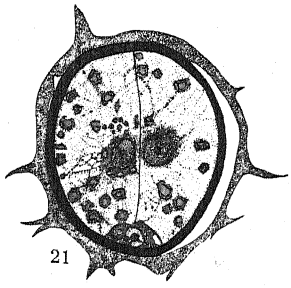

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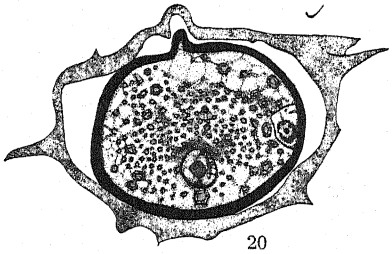

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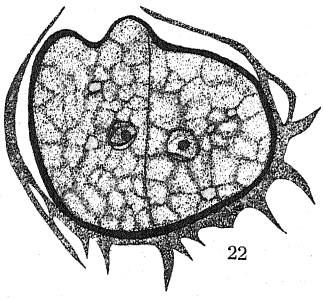

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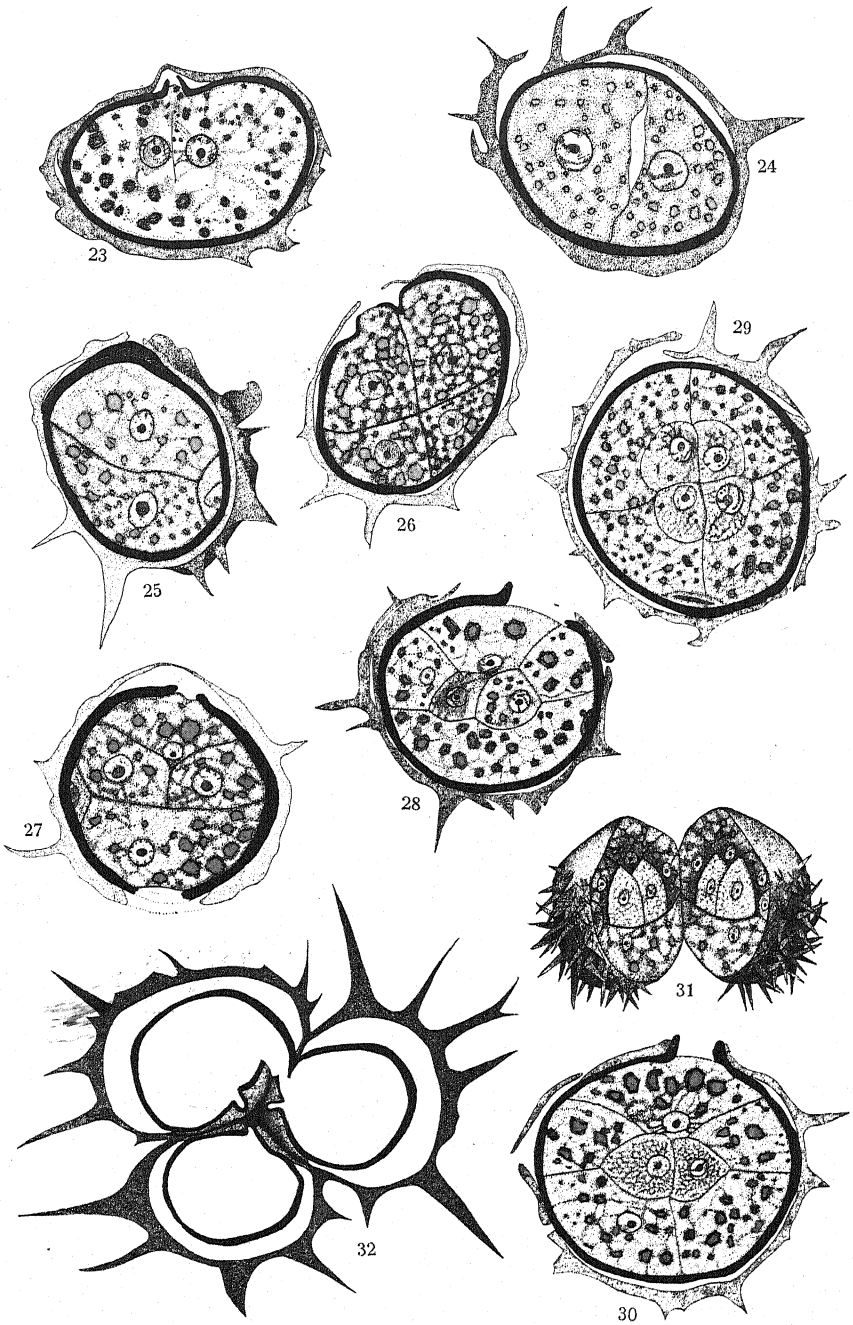
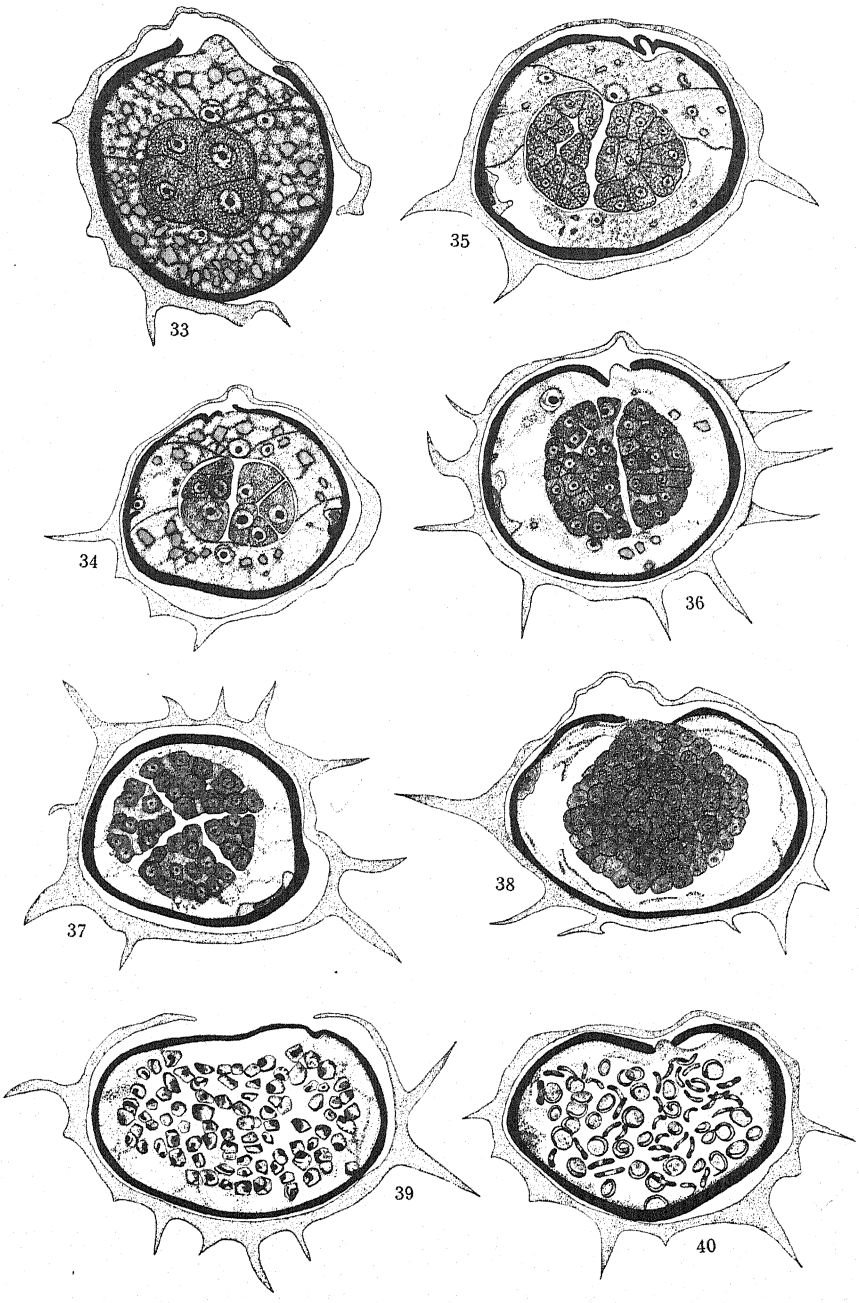
SLAGG: GAMETOPHYTE OF SELAGINELLA



SLAGG: GAMETOPHYTE OF SELAGINELLA



SLAGG: GAMETOPHYTE OF SELAGINELLA

PLATE VIII

FIG. 33. Longitudinal section in plane of first primordial wall. First division of primary spermatogenous cells.

FIGS. 34–36. Irregular multiplication of cells in spermatogenous complex. Cleavage, at right angles to plane of first primordial wall, reveals genetic groups of spermatogenous cells. Protein crystals disappear. Jacket cells disintegrate. Prothallial cell collapses.

FIG. 37. Transverse section at level of annular ridges. Cleavages reveal four genetic groups of spermatogenous cells corresponding to four primary spermatogenous cells.

FIG. 38. Genetic groups dissociated, spermatogenous cells continue to multiply, and central complex becomes spherical.

FIG. 39. Antherozoid mother cells float free in slime left by disintegrating jacket cells.

FIG. 40. Spermatogenesis practically completed.

OBSERVATIONS ON CHROMOSOME NUMBER IN *ALTHAEA ROSEA*, *CALLIRHOË INVOLUCRATA*, AND *HIBISCUS COCCINEUS*

ERNST VON KESSELER

(Received for publication August 17, 1931)

The Malvaceae are a sharply delineated family, widely distributed, with many striking characters, and with numerous forms, and would therefore seem to be excellent material for a study of taxonomic relationship from a cytological standpoint. Within the last five years, chromosome counts have been made on many species. The cottons especially, because of their economic importance, have been quite comprehensively studied cytologically by Cannon (1903), Nikolajewa (1923), Denham (1924), Youngman (1927), Beal (1928), Banerji (1929), and others.

According to the recent chromosome number list of Tischler (1927) and L. O. Gaiser (1930 *a* and *b*), the following named genera and species of Malvaceae have been investigated as to their haploid numbers. Authors are not cited, since both of these workers give comprehensive citations. The 16 or more species or forms of *Gossypium* have either 13 or 26 *n* chromosomes. The genus *Malva* is represented by three species—*M. moschata* (20), *M. palmata* (ca. 20), and *M. pusilla* (20–30). Four species of *Hibiscus* (*H. rosa-sinensis*, *H. tricuspis*, *H. tiliaceus*, and *H. surratensis*) range in haploid chromosome number from 36–39?, through 40 and 48 to 72. In *Sidalcea neo-mexicana* A. Gray $n = 13$. *Lavatera thuringiaca* has 20. In *Malvastrum capense* $n = 21$, while in *Thespesia populnea* the haploid condition reaches the lowest numbers in Malvaceae, viz. 8, 10, and 13.

The present investigation involves studies on *Callirhoë involucrata* Gray, *Hibiscus coccineus* Walt., and single and double flowered forms of the hollyhock, *Althaea rosea* Cav.

MATERIALS AND METHODS

The materials used in these investigations came from cultures and seeds belonging to The Blandy Experimental Farm.

Allen's modification of Bouin's fluid was the chief fixing fluid used. La Cour's formula 1, recommended for fixation of somatic chromosomes, was tried but gave no better results than the first mentioned fixative. The material was dehydrated, carried over to xylol, and imbedded in paraffin. Sections were cut 8 μ for both root-tip and meiotic material. Two stains were used—Heidenhain's iron-alum haematoxylin and gentian violet for the somatic material, and only the former for the meiotic. Gentian violet

proved to be somewhat better as a root-tip material stain for *Althaea rosea*, since the chromosomes in such preparations were much more sharply defined than in the haematoxylin preparations. With *Hibiscus* material this was not so apparent.

Buds were deprived of their floral envelopes before being immersed in the fixing fluid. Acetocarmine smear preparations were tried in the case of *Althaea*, in order to determine the presence of the desired stages, but this method failed for a number of reasons. The number of anthers per single androecium is very large, and there seems to be considerable variation in their time of maturity. The terminal anthers generally reach maturity first. Because of these differences, the number of desired stages, comparatively speaking, in a given bud is very small, hence the smear method does not appear very practical for this material.

The most favorable period for obtaining the desired meiotic stages in *Althaea* is during the night and early morning, 8 P.M. to 6 A.M. I found no division figures at all after 6 A.M. Root-tip material of *Althaea* shows great variation in frequency number of mitotic figures during the day, with perhaps the greatest frequencies at 6 A.M. and 4 P.M. This difference in frequency of division figures was also true of root-tip material of *Hibiscus coccineus*, with perhaps the highest number about 7 A.M. under laboratory conditions. This material was obtained by germinating the seeds in petri dishes containing moist filter paper. The meiotic material of *Callirhoë* was fixed between 5 and 6 A.M.

Observations

Althaea rosea

Counts made from heterotypic metaphases of various types of both single and double flowered forms of this species demonstrated the haploid chromosome number to be 21 (Pl. IX, figs. 1, 2). At heterotypic metaphase, in common with many other Malvaceous species, a densely staining perinuclear zone surrounds the chromatic material.

Attempts to determine the diploid chromosome number in the root-tip material were very unsatisfactory. Numerous counts of metaphase stages in somatic material of the same variety gave results varying from 38 to 50, with 48 as the most common number (fig. 3). Plate IX, figure 4, shows a plate with the theoretically expected number 42. The explanation of this variability lies in the difficulty of finding plates where splitting of some of the chromosomes has not taken place, preparatory to anaphase separation.

Callirhoë involucrata

The haploid number of this species is 12 (fig. 5), as determined from heterotypic metaphases. In this species, no perinuclear zone is present. No marked differences between the anthers as to meiotic stages occur.

Hibiscus coccineus

Attempts to count chromosomes in somatic material of this species, as in *Althaea*, were very unsatisfactory, and probably for the same reasons. Counts of metaphase plates ranged from 37 to 50. No meiotic material was obtainable.

Summary

1. The haploid chromosome number of *Althaea rosea*, as established from pollen mother cell material, is 21. This is true of both the single and double flowered varieties investigated. Observations on root-tip material do not corroborate these results, since metaphase plates gave counts ranging in number from 38 to 50, even in material from the same individual.

2. The haploid chromosome number of *Callirhoë involucrata*, as established from pollen mother cell material, is 12, the lowest number, except for *Thespesia*, yet discovered for the Malvaceae.

3. The chromosome counts in root-tip material of *Hibiscus coccineus* gave unsatisfactory results, the number per plate ranging from 37 to 50.

The writer wishes to express his thanks to Dr. Orland E. White for advice and criticism.

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EXPLANATION OF PLATE IX

The following drawings were made with a Zeiss microscope equipped with a 2 mm. achromatic objective and 30 × ocular. An Abbe camera lucida was used. The magnification of all figures is about × 4700.

Fig. 1. *Althaea rosea*, Chater's Double Yellow, polar view of 21 chromosomes at heterotypic metaphase.

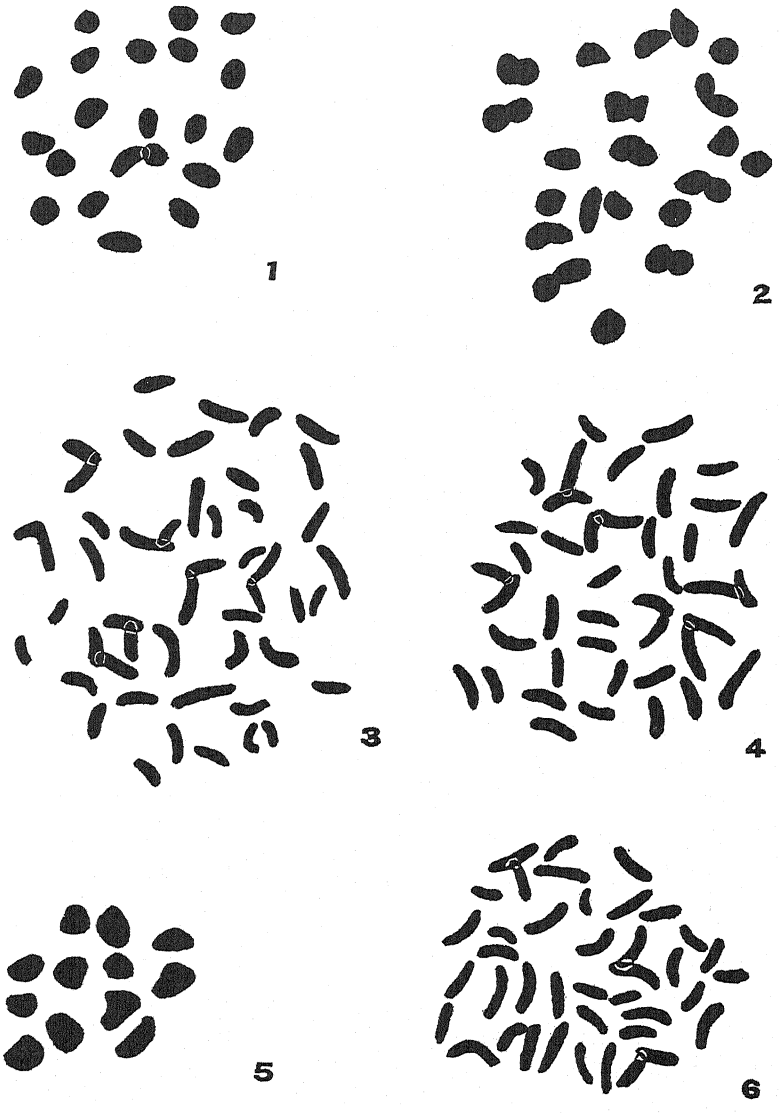
Fig. 2. *Althaea rosea*, single, polar view of 21 chromosomes at heterotypic metaphase, part of them dividing.

Fig. 3. *Althaea rosea*, Chater's Double Chamois, polar view of 48 diploid chromosomes in root-tip cells.

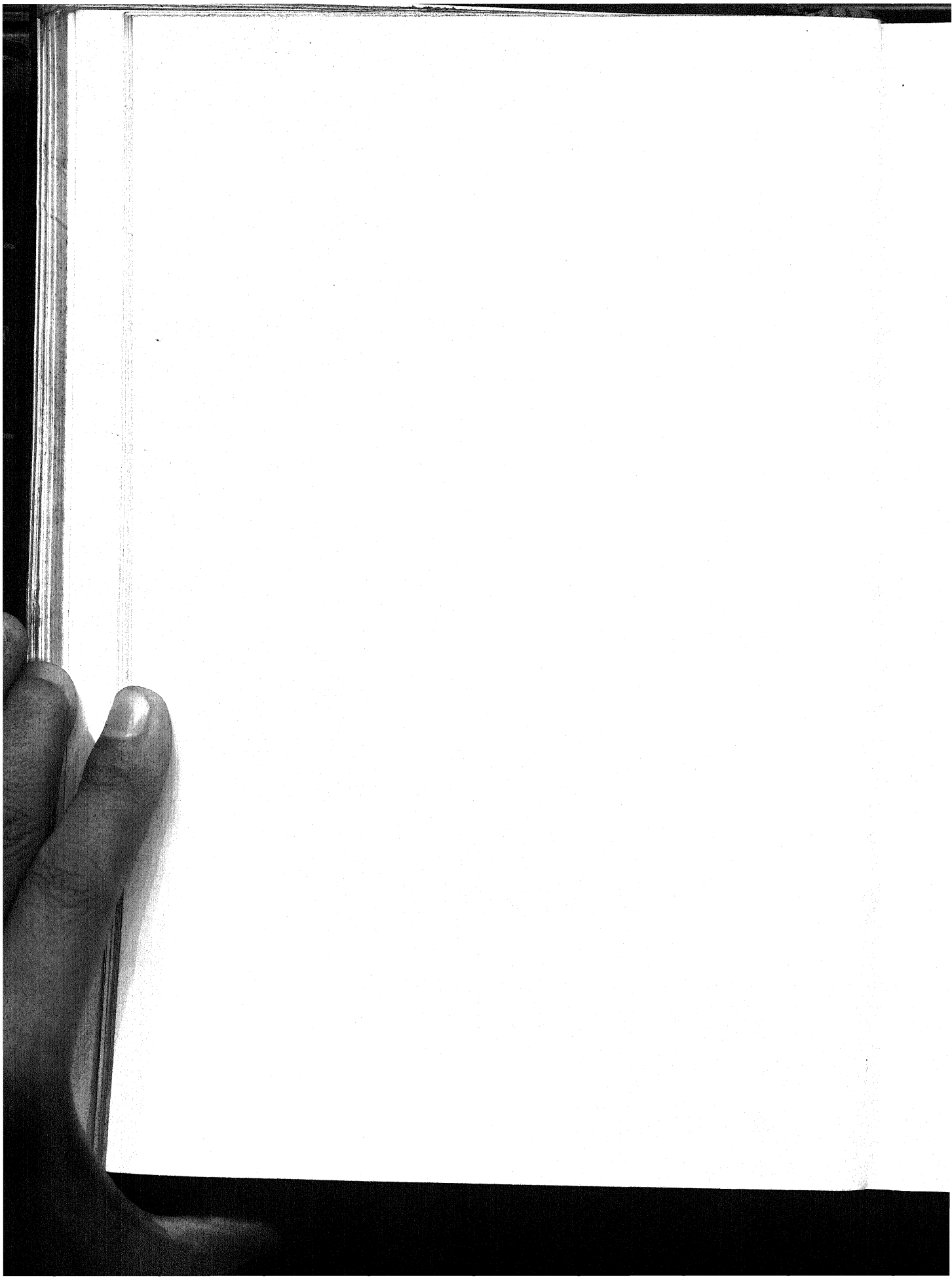
Fig. 4. *Althaea rosea*, Chater's Double Chamois, polar view of 42 diploid chromosomes in root-tip cells.

Fig. 5. *Callirhoë involucrata*, polar view of 12 chromosomes at heterotypic metaphase.

Fig. 6. *Hibiscus coccineus*, polar view of 40 diploid chromosomes in root-tip cells.



VON KESSELER: CHROMOSOME NUMBERS



MYXOMYCETES OF NORTHWESTERN OREGON

MORTON E. PECK AND HENRY C. GILBERT

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During the past twenty years the authors of this list have been accumulating, in a rather sporadic way, collections of the myxomycetes of northwestern Oregon. The number of species found has now become so large that it seems worth while to publish the list, particularly as very little has been done in Oregon hitherto in this field. The list is of course incomplete even after these years of collecting and the authors feel certain that many more species are yet to be found in the territory here included. The boundaries of the area are vague, embracing the lower, or northern, third of the Willamette valley, the coast mountains from Lincoln county north, and the west slope of the Cascades as far south as the Three Sisters mountains. Even within this area there are whole counties that have not been touched.

The list is based wholly on the collections of the authors, these aggregating some 1600 specimens. A total of 194 species have been found. Separation of varieties in many variable forms has been avoided; yet in some cases it seemed advisable to make note of varieties, so that thirteen varieties are listed. Thirty-six genera are represented and as usual the genus *Physarum* has the largest number, having forty-three species.

There are ten new species described, with illustrations of eight of the species. There are also eight species listed that have hitherto been known only from the Old World.

Some discrimination is necessary in choosing the data to be included in such a list in order to make it of most value to students of the group and still keep within the necessary limits of space. In a group having so few highly localized forms it has not seemed necessary to give more than general localities. Habitats, relative abundance, and seasonal appearance are more significant and these are always given. Some taxonomic notes are added where they have seemed pertinent. The sequence throughout is that of Macbride's "North American Slime-Moulds." The only works that have been of any great assistance in the preparation of the list are Lister's "Mycetozoa" and the above mentioned work of Macbride.

EXOSPOREAE

1. *Ceratiomyxa fructiculosa* (Muell.) Macbr. Abundant and often profuse on many kinds of rotting wood. Occasionally pink or cream colored colonies are found. Matures during spring rains, seldom found in the fall, Willamette valley, Coast slopes and foothills of the Cascades. The var. *flexuosa* List. is occasional on oak logs in the Willamette valley.

2. *C. porioides* (Alb. and Schw.) Schroet. Occasional on Douglas fir logs in the Willamette valley and foothills of the Cascades. We find this form on the same log with *C. fructiculosa* but distinct and nothing to indicate that it is only a variety of that species as Lister places it.

MYXOGASTRES

Physarales

PHYSARACEAE

3. *Fuligo cinerea* (Schw.) Morg. Rare, on old grass stems in wet places, in Nov. in the Willamette valley. This is the gregarious form.

4. *F. septica* Gml. Common all summer on old logs and stumps and on the ground. Forma *ovata* Schaeff. is most common; f. *violacea* Pers. and f. *candida* Fr. are rare, and f. *rufa* Pers. has been found but once.

5. *F. intermedia* Macbr. One fine specimen of this species has been found. It was on an old log in dense timber in the foothills of the Cascades, in July.

6. *Badhamia versicolor* List. Occasional on old oak logs in the Willamette valley during rainy weather.

7. *B. decipiens* (Curtis) Berk. Occasional on rotting wood from Nov. to Feb. in the Willamette valley.

8. *B. nitens* Berk. Occasional, usually on the bark of oak or alder logs during the winter rains in the Willamette valley. A yellow plasmodium of this species at 40° to 60° F. required 42 hours to mature.

9. *B. panicea* (Fr.) Rost. Common and occasionally profuse on old logs of various species and in brush piles during rainy weather in the Willamette valley, Coast slopes and foothills of the Cascades.

10. *B. macrocarpa* (Ces.) Rost. Rare, on the bark of oak logs in June in the Willamette valley.

11. *B. magna* Peck. Occasional on the bark of oak logs and on rotting willow in the spring in the Willamette valley. A bright yellow plasmodium required 36 hours to form sporangia at 40° to 60° F.

12. *B. foliicola* List. Found once only, in Sept. 1926, on small branches of oak in the Willamette valley.

13. *B. utricularis* (Bull.) Berk. Common and often abundant on the bark of oak logs during the rainy season in the Willamette valley and Coast slopes. The golden yellow plasmodium of this species may take two days to mature sporangia during the winter months.

14. *B. populina* List. Rare, on cottonwood logs in the Willamette valley.

15. *B. rubiginosa* (Chev.) Rost. Rare, on decaying bark of Douglas fir in late autumn in the Willamette valley.

16. *Physarum lateritium* (Berk. & Rav.) Rost. Found but once, in leaf mold under dense trees in a wet area, in Aug., in the Willamette valley.

17. *P. vernum* Somm. Rare, in old rotting weeds in Feb. in the Willamette valley. The var. *iridescens* G. List. is also rare.

18. *P. sinuosum* Weinm. Occasional and may be in large masses where found, in leaf mold in wet areas in the spring or fall in the Willamette valley.

19. *P. bitectum* List. Common in leaf mold from Nov. to June in the Willamette valley.

20. *P. diderma* Rost. Rare, on the bark of oak logs in Dec. and Jan. in the Willamette valley.

21. *P. contextum* Pers. Rare, in oak leaf mold in Nov. and Dec. in the Willamette valley.

22. *P. conglomeratum* Rost. Found but once, on a large oak log in Jan. in the Willamette valley.

23. *P. Mortoni* Macbr. Common in various kinds of leaf mold during rainy weather in the Willamette valley.

24. **P. Gilkeyanum** Gilbert. n. sp. Sporangia gregarious, globose or clavate, sessile, but narrowed at the base, 0.4 to 0.8 mm. in diameter, 0.7 to 0.9 mm. tall; peridium hyaline, grey when without lime but usually covered with a thin, even layer of greyish-white lime, brittle, breaking irregularly, the inner surface rough, the lime covering forming a reticulation of thin wrinkles over the entire sporangium; capillitium of fine hyaline threads, a dense, rigid, fine-meshed net that retains the form of the sporangium after the peridium breaks away, the lime-knots white, few, in the upper part of the sporangium small and more or less rounded, in the base of the sporangium tending to be long, branching, and somewhat badhamioid with larger meshes in the capillitium, spores dark violet in mass, globose, warted, violet brown under the lens, 9 to 11 μ in diameter.

Habitat: in leaf mold under deciduous trees and brush.

PLATE X, figure 1. *A*. Three sporangia on a bit of leaf. *B*. A small piece of capillitium from the upper part of the sporangium. *C*. A small piece of capillitium from the base of the sporangium. *D*. A single spore.

The collection on which this new species is offered was found in March, 1930 at Lebanon, Oregon, by Gilbert. There is but one colony of about seventy-five sporangia. The sporangia are very uniform in the characteristics described above. Further collections are desirable but hardly seem necessary to establish this neat and distinctive species of an already large genus. It is named for Dr. Helen Gilkey of the Oregon State College.

25. *P. cinereum* (Batsch.) Pers. Common on old logs in wet areas, in leaf mold, or on herbaceous waste, from Nov. to June, in the Willamette valley.

26. **P. lepidoideum** Gilbert. n. sp. Sporangia sessile, solitary or in small groups on black waste matter forming a small hypothallus, irregularly globose, 0.5 to 1.2 mm. in diameter, with a tendency to form small crowded plasmodiocarps; peridium blue-grey, membranous, iridescent, with a thin layer of flat, more or less rounded lime scales similar to *Lepidoderma;* capillitium a dense net of colorless threads, the limeknots white, conspicuous but not abundant, medium sized, rounded or with points along the capillitial threads, a few sometimes larger and more angled; spores dark purple in mass,

globose, under the lens bright violet, uniformly finely warted, 12 to 14 μ in diameter.

Habitat: On old kale stalks in a pile of herbaceous waste.

PLATE X, figure 2. *A*. Several sporangia on a small piece of kale stalk. *B*. A small piece of the capillitium. *C*. A single spore.

The collection on which this new species is offered was found in January, 1928 at Salem, Oregon, by Gilbert. The Lepidoderma-like peridium is distinctive. The spores are larger than in most Physarums and very uniform in size.

27. *P. virescens* Ditm. Rare, on decayed oak wood in May in the Willamette valley.

28. **P. nudum** Macbr. n. sp. Sporangia sessile, globose or somewhat plasmodiocarpous, occasionally with a pallid, slender, usually prostrate stipe, 0.4 to 0.7 mm. in width; peridium single, limeless, dark grey, somewhat iridescent; hypothallus scanty, reticulate, membranous, hyaline, connecting the lines of sporangia; columella none; capillitium sparse and nearly or quite limeless, composed of slender threadlike tubules here and there expanded into hyaline vesicular structures; spores violet black in mass, clear violet under the lens, minutely roughened, 9.5 to 11 μ in diameter.

A specimen collected by T. H. Macbride, probably in Washington, and the data now lost, was marked as possibly a new species. Then a specimen collected by Gilbert in May, 1930, near Salem, Oregon, proved to be the same. The species is described under Dr. Macbride's name. In its limeless character it suggests *P. confertum* but differs in size, color, and habit of growth; suggesting also limeless forms of *P. didermoides* but different in habit, and with smaller, lighter colored, much smoother spores. Mr. Gilbert notes that the sporangia are milk white when young, which would indicate a watery or white plasmodium. Apparently the plasmodium remains in the soft rotting wood, coming to the surface only at the time of fruiting.

29. *P. melleum* Mars. Rare, in leaf mold around oak logs in Nov. in the Willamette valley.

30. *P. citrinum* Schum. Rare, on old logs in wet areas in June in the Willamette valley.

31. **P. perfectum** M. Peck n. sp. Sporangia loosely gregarious, greyish white, globose, stipitate, 0.6 to 0.8 mm. in diameter; hypothallus very thin, colorless, wide-spreading; stipe yellowish white, stout, calcareous, nearly smooth, slightly narrowed upward, equaling or a little surpassing the diameter of the sporangium; columella well developed, white, conical, nearly one-third the height of the sporangium; peridium a thin membrane, evenly granular with included lime and thickly sprinkled with round, mainly superficial white scales of lime; capillitium moderately dense with abundant rounded and elongated, pale yellowish calcareous nodes; spores minutely roughened, violaceous brown, 10 to 11 μ in diameter.

Habitat: The decaying bark of *Populus* sp.

PLATE XI, figure 3. *A*. Habit sketch. *B*. Diagrammatic sketch of the columella. *C*. A portion of capillitium. *D*. A spore.

Seemingly a well marked species, having a trim, finished appearance. Two or three colonies have been found in the neighborhood of Salem, Oregon. The most striking structural feature is the strongly developed columella; this together with the habit, length of stipe, and several other features suggest *P. melleum* (Berk. & Br.) Mass. but the color and greater size at once distinguish it.

32. *P. globuliferum* (Bull.) Pers. Rare, on old logs in June in the Willamette valley.

33. *P. murinum* List. Rare, on decayed logs in Nov. in the Willamette valley.

34. *P. penetrale* Rex. Rare and few in one place on old logs in dense woods in May and June and in Nov. in the Willamette valley.

35. *P. luteo-album* List. Rare, in the high Cascades in summer.

36. *P. nucleatum* Rex. One old colony was found on a log along the Salmon river on the Coast slopes.

37. *P. Newtoni* Macbr. One large colony found on decaying wood in June on the Three Sisters mountains.

38. *P. psittacinum* Ditm. Rare, in leaf mold in June in the Willamette valley.

39. *P. leucophaeum* Fr. Common, usually on oak, from Nov. to June in the Willamette valley.

40. *P. nodulosum* Cke. & Balf. Rare, on alder logs in wet areas in Dec. in the Willamette valley.

41. *P. didermoides* (Pers.) Rost. One profuse mass found on a dung pile by a barn in Sept. in the Willamette valley. The var. *lividum* Rost. seems to be rare, as a few only have been found, on potatoes in a bin in the Willamette valley.

42. *P. leucopus* Link. Rare, on alder and oak logs during spring or fall rains in the Willamette valley.

43. *P. compressum* A. & S. Occasional on oak wood and in herbaceous waste in Jan. and Sept., in the Willamette valley.

44. *P. straminipes* List. One colony only, found in leaf mold in July in the Willamette valley. We believe this to be the first report of this species in North America.

45. *P. notabile* Macbr. Common and variable, in moss and on old logs from Dec. to May in the Willamette valley.

46. *P. nicaraguense* Macbr. Found once only, in moss on an oak log in Dec. in the Willamette valley.

47. *P. carneum* G. List & Sturgis. Occasional and few in one place, in leaf mold and on wood, from Dec. to May in the Willamette valley. The plasmodium, as far as noted, is white.

48. *P. citrinellum* Peck. Occasional on rotting oak and in leaf mold under pines, from Nov. to June in the Willamette valley.

49. *P. albescens* Ellis. Rare, on living twigs of a dwarf *Vaccinium* in July on the Three Sisters mountains.

50. *P. variabile* Rex. Collected once only, in leaf mold of a swampy woods in June in the Willamette valley.

51. *P. auriscalpium* Cooke. Occasional in leaf mold, moss, or on oak wood from June to Oct. in the Willamette valley and foothills of the Cascades.

52. *P. galbeum* Wing. Rare, on old logs in June in the Willamette valley.

53. *P. tenerum* Rex. Rare, in moss or on logs in wet areas in Dec. in the Willamette valley.

54. **P. simplex** M. Peck. n. sp. Sporangia stipitate, globose or a little depressed, minute, 0.2 to 0.3 mm. in diameter, very dark or sometimes dull yellowish; stipe 0.5 to 2 mm. long, slender, narrowed upward, irregularly grooved, often very crooked and drooping above, light yellowish, little or not at all calcareous; hypothallus none; peridium thin, iridescent, often strewn with abundant flakes of lime but sometimes nearly limeless, then appearing black, persistent below; columella none; capillitium of very delicate threads springing from the peridial floor, with a few small yellowish-grey calcareous nodes or none and almost without dilations at the intersections; spores violaceous brown, minutely roughened, 7 to 9 μ in diameter.

Habitat: dead bark of *Pseudotsuga*.

PLATE XI, figure 4. *A*. Habit sketch. *B*. Diagram of the capillitium. *C*. A spore.

A very delicate, minute species found twice in the vicinity of Salem, Oregon. It is related to *P. flavicomum* Berk., differing in the longer stipe, the more or less calcareous peridium, and the less dense capillitium with very few calcareous nodes.

55. *P. nutans* Pers. Common and often profuse on all sorts of logs and in leaf mold at all times of the year in the Willamette valley and on the Coast slopes.

56. *P. viride* (Bull.) Pers. Common and variable, on many kinds of logs and in leaf mold, most abundant in May and June in the Willamette valley.

57. *Craterium aureum* (Schum.) Rost. Rare, in leaf mold in the spring in the Willamette valley.

58. *C. leucocephalum* (Pers.) Ditm. Common in leaf mold of wet areas in the spring or fall in the Willamette valley. One large colony of var. *scyphoides* List. was found in maple leaf mold.

59. *C. minutum* (Leers) Fr. Common in leaf mold during the rainy season in the Willamette valley. We find great variation in our collections. Lister, in the 3rd ed. of "Mycetozoa," notes that a number of forms arise from the same plasmodium. We find variations in form and color in different collections but not within single groups all of which might be from one

plasmodium. We also find red as well as yellow plasmodia. Our material suggests more than one species. Further collection and more detailed study are necessary on our western forms, we believe.

60. *Leocarpus fragilis* (Dicks.) Rost. Common and sometimes profuse from June to Dec. in leaf mold in the Willamette valley and foothills of the Cascades. The color varies from tan to dark red that is almost black.

DIDYMIACEAE

61. *Musilago spongiosa* (Leyss.) Morg. Rare, but in large masses where found. It spreads over grass, herbs, and tree trunks in dense wet areas from May to Aug. in the Willamette valley.

62. *Didymium anellus* Morg. Rare, in leaf mold during winter rains in the Willamette valley.

63. *D. Wilczekii* Meyl. Occasional on old weeds and corn stalks in the spring in the Willamette valley and in the summer in the Cascades.

64. *D. squamulosum* Fr. Common and often abundant in leaf mold and on old weeds all the year round in the Willamette valley. The lime crystals may be few or in heavy folds over the sporangium.

65. *D. melanospermum* (Pers.) Macbr. Occasional in moss or leaf mold under dense timber from June to Aug. in the Willamette valley and on the Coast slopes.

66. *D. minus* List. Rare, in moss during the winter rains in the Willamette valley.

67. *D. clavus* Rab. Common any time of the year in leaf mold and on rotting weeds, but few in one place, in the Willamette valley.

68. *D. nigripes* (Link.) Fr. One large colony found in the leaf mold of a wet area in May in the Willamette valley.

69. *D. xanthopus* (Ditmar) Fr. Common on old weeds and corn stalks in the spring in the Willamette valley. In 1931 profuse colonies of a form that varies from the typical *xanthopus* were found on madrona leaf mold. The plasmodium of this form is clear yellow. The spores are larger and the columella less definite than in *xanthopus*. Further study must be made of it.

70. *D. difforme* Dby. Common and often abundant on old weeds and in leaf mold, from Nov. to May, in the Willamette valley.

71. *D. trachisporium* List. This form has been found a number of times in Mr. Gilbert's garden about four inches under ground on the surface of daffodil bulbs in the summer months. We believe this is the first report of this species in North America. The habitat would suggest that it had been imported on the daffodil bulbs from Holland.

72. *Diderma reticulatum* (Rost.) Morg. Rare, on decaying leaves of pond lily in the autumn in the Willamette valley.

73. *D. spumarioides* Fr. Usually in large masses, coming out of the ground, but not common; in the spring in the Willamette valley and foothills of the Cascades.

74. *D. globosum* Pers. Rare, in leaf mold of mixed woods in March in the Willamette valley.

75. *D. crustaceum* Peck. Rare, in leaf mold of deciduous woods in March in the Willamette valley.

76. *D. niveum* Macbr. Rare, on rotting wood in June on the Three Sisters mountains.

77. *D. cinereum* Morg. Rare, on the ground under old wood in Feb. in the Willamette valley.

78. *D. hemisphericum* (Bull.) Horne. In moss on oak logs in the fall in the Willamette valley. Sometimes abundant on piles of old leaves in the spring.

79. *D. ochraceum* Hoffm. Rare, on decaying willow wood in July in the Willamette valley.

80. *D. Trevelyani* Fr. Occasional and usually profuse where found, in leaf mold in the spring and fall in the Willamette valley.

81. *D. radiatum* List. Common on many kinds of wood or in leaf mold during fall and winter rains in the Willamette valley. Occasional in the spring. The var. *umbilicatum* Meyl. is rare.

82. *D. asteroides* List. Occasional in madrona leaf mold and on old pine needles in the spring in the Willamette valley.

83. *Lepidoderma tigrinum* (Schrad.) Rost. Rare, on well rotted wood during winter rains in the Willamette valley.

84. *L. caristianum* (Rabenh.) Rost. Rare, on fallen twigs, leaves, and bits of wood close to snow banks in June on the Three Sisters mountains.

Stemonitales

Amaurochaetaceae

85. *Amaurochaete fuliginosa* Macbr. Occasional in the fall in the Willamette valley.

86. *A. tubulina* (A. & S.) Macbr. Found but once, in Aug., in the Willamette valley.

Stemonitaceae

87. *Brefeldia maxima* (Fr.) Rost. Rare, on rotting wood during the summer in the Willamette valley.

88. *Stemonitis trechispora* (Berk.) Torr. Rare, on oak stumps in June in the Willamette valley.

89. *S. fusca* (Roth) Rost. Common in small masses, usually on oak logs during spring or fall rains in the Willamette valley.

90. *S. dictyospora* Rost. Rare, but in large colonies, on oak and alder logs in June in the Willamette valley.

91. *S. nigrescens* Rex. Rare, on the under side of old pine logs in May in the Willamette valley.

92. *S. virginiensis* Rex. Rare, on well rotted wood in June in the Willamette valley.

93. *S. hyperopta* Meyl. Rare, on old logs in wet areas, June to Aug., in the Willamette valley. The var. *microspora* List. is also rare and is found on old logs in river bottom land in May and June. We believe this to be the first report of this variety for North America.

94. *S. Webberi* Rex. Rare, found but twice, in May and June, on old pine logs in the Willamette valley.

95. *S. splendens* Rost. Occasional on oak wood in April in the Willamette valley.

96. *S. Smithii* Macbr. Occasional on well rotted wood in May in the Willamette valley. Our specimens are up to 6 mm. rather than less than 3 mm. as given by Macbride for the type.

97. *S. axifera* (Bull.) Macbr. Frequent on rotting logs during spring or fall rains in the Willamette valley and on the Coast slopes. The plasmodium of some of our specimens is yellow. We find that the spores of some specimens are small, being but 4 to 5 μ, suggesting *S. Smithii*, but the sporangia 10 mm. tall.

98. *S. flavogenita* Jahn. Common on old logs from Oct. to Dec. in the Willamette valley. We find that the plasmodium is often white and not yellow as the name implies.

99. *S. pallida* Wing. Rare, on well rotted alder in May in the Willamette valley.

100. *S. herbatica* Peck. Occasional during spring rains in the Willamette valley. A lemon yellow plasmodium found in leaf mold under dense Douglas fir trees developed a small form (2 to 4 mm.) of this species.

101. *Comatricha caespitosa* Sturgis. Found but once, on the decaying trunk of Mountain Hemlock in June on the Three Sisters mountains.

102. *C. flaccida* List. Two light yellow masses of young Stemonitis type were found on a willow stump near the Willamette river. When collected after maturing these proved to be this species, except that the hypothallus is silvery and not brown as given by Macbride in N. A. S. M.

103. *C. longa* Peck. Rare, on willow logs near the Willamette river in June.

104. *C. irregularis* Rex. Found but once on the dead bark of Lodgepole Pine in July on the Three Sisters mountains and once on an old log in the Willamette valley.

105. *C. Suksdorfii* Ell. & Evh. Plentiful on decaying trunks of Mountain Hemlock in June and July on the Three Sisters mountains. Two collections from the Three Sisters area seem nearest to this species yet differ in several characters. Further study will determine whether this form is specific or not.

106. **C. pacifica** Macbr. n. sp. Published as a var. of *C. aequalis* Peck in N. A. Slime-Molds, 2 ed.: 181, 1922, and now given specific rank.

Sporangia gregarious, erect, deep violaceous, cylindrical or slightly ovate, 7–8 mm. tall, stipitate; stipe about half the total height, black, pol-

ished, even; hypothallus dark brown, shining, conspicuous, more or less distinct for each sporangium, often connected in plasmodiocarpous fashion but not continuous; columella dark, tapering, attaining almost the summit of the sporangium; capillitium dense, of dark flexuose threads, branching repeatedly and forming an intricate network, the free ends numerous, short, little if any paler than the threads from which they arise; spores dark violaceous, distinctly warted, 9.5–11 μ in diameter.

Resembling *C. aequalis* Peck, of which it was formerly regarded as a variety, but larger, darker, more distinctly purple, with larger spores and a different hypothallus.

Found on the dead bark and twigs of mountain hemlock on the Three Sisters mountains. Collected twice, the first time in July, 1914.

107. *C. nigra* (Pers.) Schroet. Common and more abundant in the higher altitudes on many kinds of wood and at all seasons in the Willamette valley. Found everywhere in our territory.

108. *C. aequalis* Peck. Rare, on logs in wet areas during the rainy season in the Willamette valley.

109. *C. typhoides* (Bull.) Rost. Common and often profuse, usually on oak or willow, during the rainy season in the Willamette valley, and on the Coast slopes and foothills of the Cascades.

110. **C. reticulata** Gilbert n. sp. Sporangia dark lilac-brown, in small loose tufts, erect, on a thin silvery hypothallus, 1.5 to 3 mm. tall, short cylindric and obtuse at both base and apex; stipe about one-half the height, black, shining; columella dissipating in the upper one-fourth of the sporangium; primary branches of the capillitium numerous, often thickened near the columella; these branching and anastomosing to form an irregular net toward the surface, which retains the form of the sporangium after spore dispersal, the free tips outside the net short and abundant; spores pale lilac-brown, strongly reticulate with meshes of irregular size, 7 to 9 μ in diameter.

Habitat: On rotting coniferous wood.

PLATE XII, figure 5. *A*. A small tuft of sporangia. *B*. A single sporangium in longitudinal section. *C*. Three spores.

This new species is based on a collection made by Gilbert in May, 1930, on pine logs at Turner, Oregon. The network of the capillitium suggests *Stemonitis* but is decidedly not on the surface as is shown by the many free tips outside the net. The most striking characteristic of this new species is the beautifully reticulate, small sized spores.

111. *C. laxa* Rost. Occasional on rotting willow and alder during spring rains in the Willamette valley.

112. *C. elegans* (Racib.) List. Rare, on oak logs in the spring in the Willamette valley.

113. *C. rubens* List. One small colony found maturing in oak leaf mold in March in the Willamette valley.

114. *C. pulchella* (Bab.) Rost. Occasional on the bark of oak logs and

on pine needles from Dec. to April in the Willamette valley. The var. *gracilis* List. has been found once, on a Douglas fir log in April in the Willamette valley.

LAMPRODERMACEAE

115. *Enerthenema papillatum* (Pers.) Rost. Common on old wood and bark of various kinds during the rainy season in the Willamette valley.

116. *Lamproderma violaceum* (Fr.) Rost. Common and often abundant in moss and on wood or bark of many kinds of logs from Nov. to April in the Willamette valley and on the Coast slopes. The var. *carestiae* List. is rare.

117. *L. columbinum* (Pers.) Rost. Common in dense woods on old logs, usually Douglas fir, from Nov. to June in the Willamette Valley and in the Cascade mountains.

118. *L. scintillans* (Berk. & Br.) Morg. Common from Oct. to April, usually in leaf mold or may be on logs in the Willamette valley and on the Coast slopes.

119. *L. arcyrionema* Rost. Found once only, on the underside of a large log in June in the Willamette valley.

120. *L. atrosporum* Meyl. Found but once, on dead but sound wood of Mountain Hemlock in June on the Three Sisters mountains.

121. *L. physaroides* (A. & S.) Rost. Rare, on decaying wood in the spring in the Willamette valley.

122. *L. robustum* Ell. & Evh. Rare, on mossy logs in the Cascades during the summer.

Cribrariales

LICEACEAE

123. *Licea variabilis* Schrad. Rare, on Douglas fir logs in Dec. and Jan. in the Willamette valley.

124. *L. minima* Fr. Rare but may be profuse where found, on old Douglas fir logs in the summer in the Willamette valley.

125. *L. tenera* Jahn. Rare, a few sporangia only found on old wood in March in the Willamette valley. This material differs from the description only in that the sporangia are larger. We know of no other report of this species from North America.

TUBIFERACEAE

126. *Lindbladia effusa* (Ehr.) Rost. Occasional on logs or stumps from April to Aug. in the Willamette valley. The var. *simplex* Rex has been collected once, on an old Douglas fir log in a wet area in the Willamette valley.

127. *Tubifera ferruginosa* (Batsch.) Macbr. Common on rotting logs from Dec. to June in the Willamette valley and foothills of the Cascades.

RETICULARIACEAE

128. *Reticularia Lycoperdon* (Bull.) Rost. Occasional on old logs from April to Aug. in the Willamette valley and foothills of the Cascades.

129. *Enteridium splendens* Morg. Rare, on old logs in May in the Willamette valley.

130. *E. olivaceum* Ehr. Occasional on rotting Douglas fir, pine, or oak, never abundant, during winter rains in the Willamette valley and in summer in the Cascades.

131. *Dictydiaethalium plumbeum* (Schum.) Rost. Occasional on old wood in the spring or fall in the Willamette valley.

132. *Liceopsis lobata* Torrend. Occasional on large Douglas fir logs under loose bark. This is the flat aethalial form. Found in May and June in the Willamette valley.

CRIBRARIACEAE

133. *Cribraria argillacea* Pers. Common on well rotted logs from Dec. to June in the Willamette valley and in the higher Cascades.

134. *C. macrocarpa* Schrad. Common in the spring and fall on Douglas fir logs in the Willamette valley.

135. *C. aurantiaca* Schrad. Common, and at times abundant on all sorts of old wood from Dec. to June in the Willamette valley and on the Coast slopes.

136. *C. rufa* (Roth) Rost. Common during spring rains on old Douglas fir logs, but few in any one place, in the Willamette valley and on the Three Sisters mountains.

137. *C. splendens* (Schrad.) Rost. Occasional on Douglas fir logs in the summer in the Willamette valley and foothills of the Cascades.

138. *C. dictydioides* Cke. & Balf. Occasional on well rotted Douglas fir during rainy season in the Willamette valley.

139. *C. minutissima* Schw. Rare, in the spring on well rotted wood in the Willamette valley.

140. *C. tenella* Schrad. Rare, on old Douglas fir logs in the spring in the Willamette valley and on the Coast slopes.

141. **C. oregana** Gilbert n. sp. Sporangia scattered or gregarious, globose, brown, small, .2 to .4 mm. in diameter, erect or nodding on subulate wrinkled stipes; stipe dark brown to black toward the base and .5 to 1.2 mm. long; hypothallus none; calyculus one-third to one-half the sporangium, the margin toothed and irregular, without ribs or veins but rough with plasmodic granules; nodes of the net variable, usually having several large, irregular, branched, and somewhat thickened dark nodes with numerous irregular smaller nodes, the connecting threads brown or nearly colorless, free ends few, and the size of the meshes variable; spores yellow-brown in mass, pale ochraceous under the lens, the surface smooth but appearing rough from the spore contents, globose, 8 to 9.5 μ diameter.

Habitat: Well rotted Douglas fir wood.

PLATE XII, figure 6. *A*. Habit sketch of sporangia. *B*. Enlarged drawing of the nodes and threads. *C*. Four spores.

This form has been collected several times in the last few years by Peck and Gilbert. It is found on well rotted Douglas fir logs during May and June in the Willamette valley. It differs most strikingly from other Cribrarias in the very large spores. With a hand lens the small sporangium with large irregular dark nodes is definitive. These nodes are larger in comparison with the size of the sporangium than in any other *Cribraria*. The lack of ribs or veins on the calyculus should also be noted.

142. *C. elegans* Berk. & C. Rare, in late fall on Douglas fir logs in the Willamette valley.

143. *C. purpurea* Schrad. Rare but sometimes found in large colonies on decaying coniferous logs in late fall in the Willamette valley and lower Cascades.

144. *C. piriformis* Schrad. Common on old logs, usually Douglas fir, in the spring in the Willamette valley.

145. *Dictydium cancellatum* (Batsch.) Macbr. Common and at times abundant on well rotted Douglas fir in the spring and fall in the Willamette valley and foothills of the Cascades. The var. *purpureum* Macbr. is found occasionally.

Lycogalales

LYCOGALACEAE

146. *Lycogala epidendrum* (Buxb.) Fr. Common on all kinds of logs from Oct. to June in the Willamette valley and Coast slopes.

147. *L. exiguum* Morg. One large colony was found on the under side of a Douglas fir log in June in the foothills of the Cascades.

Trichiales

DIANEMACEAE

148. *Margarita metallica* (Berk. & Br.) List. Occasional on old logs from Dec. to May in the Willamette valley.

149. *Dianema depressum* List. This species has now been found twice on small rotting branches in wet areas in the Willamette valley. We believe this to be the first report of this species in North America.

150. *D. corticatum* List. Common during winter rains on old wood of various kinds, in the Willamette valley and Cascades. The plasmodium is tan colored.

PERICHAENACEAE

151. *Ophiotheca vermicularis* (Schw.) Macbr. Common on old weeds and grass from Jan. to April in the Willamette valley.

152. *Perichaena corticalis* (Batsch.) Rost. Common and often profuse during rainy weather on the bark of old logs of ash, alder, and oak in the Willamette valley.

153. *P. marginata* Schw. Rare, on oak bark in Nov. in the Willamette valley.

ARCYRIACEAE

154. *Lachnobolus congestus* List. Rare, found in wet areas hanging loosely from the under side of logs from Nov. to May in the Willamette valley.

155. *L. occidentalis* Macbr. Found but once, on an oak log in Jan. in the Willamette valley.

156. *Arcyria magna* Rex. Collected but once, in Jan. on rotting Douglas fir in the Willamette valley.

157. *A. Oerstedtii* Rost. Rare, on rotting wood on the Coast slopes or in the Willamette valley during the fall rains.

158. *A. nutans* (Bull.) Grev. Common but not abundant on logs during spring and fall rains in the Willamette valley, on the Coast slopes and foothills of the Cascades.

159. *A. virescens* G. List. One fine colony has been found, on a log in June in the foothills of the Cascades. This seems easily distinguished from *A. nutans*. We believe this to be the first report of this species for North America.

160. *A. versicolor* Phillips. Rare in the valley but common at high altitudes in leaf mold or on logs. Very abundant on the Three Sisters mountains.

161. *A. incarnata* Pers. Common during rains on all sorts of rotting wood in the Willamette valley, on the Coast slopes, and in the Cascades. Occasionally light red or rose colored colonies are found.

162. *A. nodulosa* Macbr. Occasional during winter rains on oak logs in the Willamette valley.

163. *A. ferruginea* Sauter. One specimen only has been found, in late Nov. in leaf mold in the Willamette valley.

164. *A. denudata* Shl. Occasional during spring and fall rains on well rotted wood in the Willamette valley.

165. *A. cinerea* (Bull.) Pers. Common but never abundant, on wet logs of many species all the year in the Willamette valley. Occasionally pure white specimens are found.

166. *A. pomiformis* (Leers) Rost. Common, but few and scattered on any one log, from Nov. to May in the Willamette valley.

167. *A. insignis* Kalchbr. & Cke. Occasional on logs in the spring in the Willamette valley.

PROTOTRICHIACEAE

168. *Prototrichia metallica* (Berk.) Mass. Rare and few in one place, on old logs during winter rains in the Willamette valley and high Cascades.

TRICHIACEAE

169. *Hemitrichia Karstenii* Rost. Rare, found on well rotted oak wood in April in the Willamette valley and also in the Cascades.

170. *H. ovata* (Pers.) Macbr. Rare and few in one place during winter rains in the Willamette valley.

171. *H. leiocarpa* (Cke.) Macbr. Found but once, on an old cherry stump in the Willamette valley.

172. *H. clavata* Rost. Common and often abundant on old logs during rainy weather in the Willamette valley and on the Coast slopes.

173. *H. stipitata* (Mass.) Macbr. Rare, during rains on well rotted wood in the Willamette valley.

174. *H. leiotricha* List. One specimen found, in moss on an old log in the foothills of the Cascades in June. We believe this to be the first report of this species for North America.

175. *H. vesparium* (Batsch.) Macbr. Rare, on old logs in wet areas in June in the Willamette valley. As found here the capillitium is of separate threads and not forming a net.

176. *H. stipita* (Schw.) Macbr. Rare, on rotting oak in Dec. in the Willamette valley.

177. *Trichia inconspicua* Rost. Common on bark of various kinds from Dec. to April in the Willamette valley.

178. *T. contorta* Rost. Common on bark of old logs, such as apple, alder, ash, and oak from Dec. to April, in the Willamette valley.

179. **T. Macbridei** M. Peck. n. sp. Plasmodium bright pink; sporangia closely gregarious or crowded on a strongly developed, dark hypothallus, purplish brown to dark ochraceous, sessile, depressed globose, oval or forming short curved or annular plasmodiocarps; peridium somewhat iridescent, thin, translucent but finely granular, breaking irregularly; capillitium and spore-mass ochraceous; elaters very long to very short in the same sporangium, simple, uneven, abruptly contracted to simple or bifurcate tips, marked throughout with scattered blunt spines and warts, the spirals mostly 3 to 4, imperfectly developed, sometimes broken and replaced for a distance with half-rings; spores finely and closely spinulose, 11 to 13 μ in diameter.

Habitat: Decaying logs of *Populus trichocarpa*.

PLATE XIII, figure 7. *A*. Habit sketch. *B*. Typical elaters. *C*. A spore.

This species has been found several times at Salem, Oregon, in mid-winter, sometimes in very large colonies. The plasmodium emerges for fructification bright pink, retaining this color till the final changes to maturity begin. The relationship is with *T. contorta* (Ditm.) Rost., a species already sufficiently confused. The pink plasmodium, large sporangia, strongly developed hypothallus, and details of capillitial structure distinguish it. The species is dedicated to Dr. T. H. Macbride.

180. *T. varia* Pers. Abundant everywhere on all sorts of wood and in leaf mold and at all times of the year.

181. **T. cascadensis** Gilbert. n. sp. Sporangia scattered or loosely clustered, sessile, globose or forming short plasmodiocarps, 1 mm. in diameter and 1 to 4 mm. long, dull black, with no hypothallus; peridium single, thick,

granular, dull black on both surfaces, breaking irregularly to expose the yellow spores and capillitium; elaters numerous, long, 7 to 8 μ in diameter, the spiral bands thin, uneven, and closely wound, 3 to 5, covered with many fine warts which are most numerous along the edges of the bands, the tips of the elaters rounded, without enlargements, and entirely covered with the warted spiral bands, each elater with a conspicuous granular core; spores globose, yellow in mass, almost colorless under the lens, minutely and unevenly warted, 14 to 16 μ in diameter.

Habitat: On old *Thuja* logs in dense timber in the Cascades.

PLATE XIII, figure 8. *A*. Two sporangia on a small bit of wood. *B*. Enlarged tip of an elater, to show the core and warted spiral bands. *C*. Three spores.

The specimen on which this species is based was found in dense timber in the foothills near Mount Jefferson on July 5. All of the sporangia are well matured and in good condition. It is nearest to *T. alpina* Meyl., but differs so definitely that it seems reasonable to consider it a separate species. The elaters are larger than those of *T. alpina* and unlike those of any other *Trichia*. The bands, core, and tip of the elaters are distinctive. The simple peridium is quite different from the distinctly double peridium of *T. alpina*. We offer this as a new *Trichia* named for the Cascade mountains of Oregon.

182. *T. lutescens* List. Found but once, on an old log in a wet area in Nov. in the Willamette valley. We believe this to be the first report of this species in North America.

183. *T. persimilis* Karst. Rare, on logs on the Coast slopes in Dec.

184. *T. favoginea* (Batsch.) Pers. Common and variable, on old wood from Oct. to June in the Willamette valley and foothills of the Cascades.

185. *T. scabra* Rost. Common on old wood from Dec. to May in the Willamette valley and on the Coast slopes.

186. *T. verrucosa* Berk. Rare, on old Douglas fir logs in May and June in the Willamette valley and foothills of the Cascades.

187. *T. Botrytis* Pers. Common on many kinds of old wood and at all times of the year in the Willamette valley and on the Coast slopes. The shape and color of the sporangia and the elaters vary greatly. Numerous varieties might be listed for this unstable species here in Oregon.

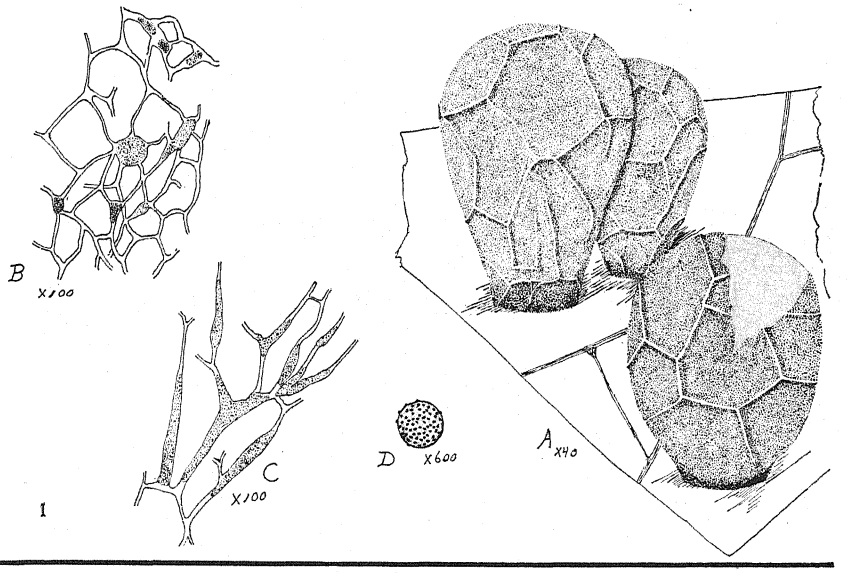
188. *T. subfusca* Rex. Rare, in leaf mold under pines in May and on old logs in dense timber in the Willamette valley and foothills of the Cascades.

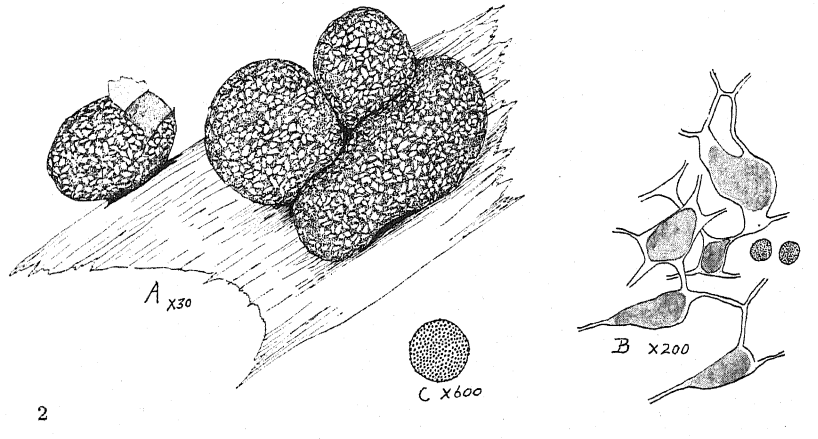
189. *T. erecta* Rex. A single colony was found on bits of wood close to the snow in June on the Three Sisters mountains.

190. *T. decipiens* (Pers.) Macbr. Common and often abundant on old logs, usually Douglas fir, at all times of the year in the Willamette valley.

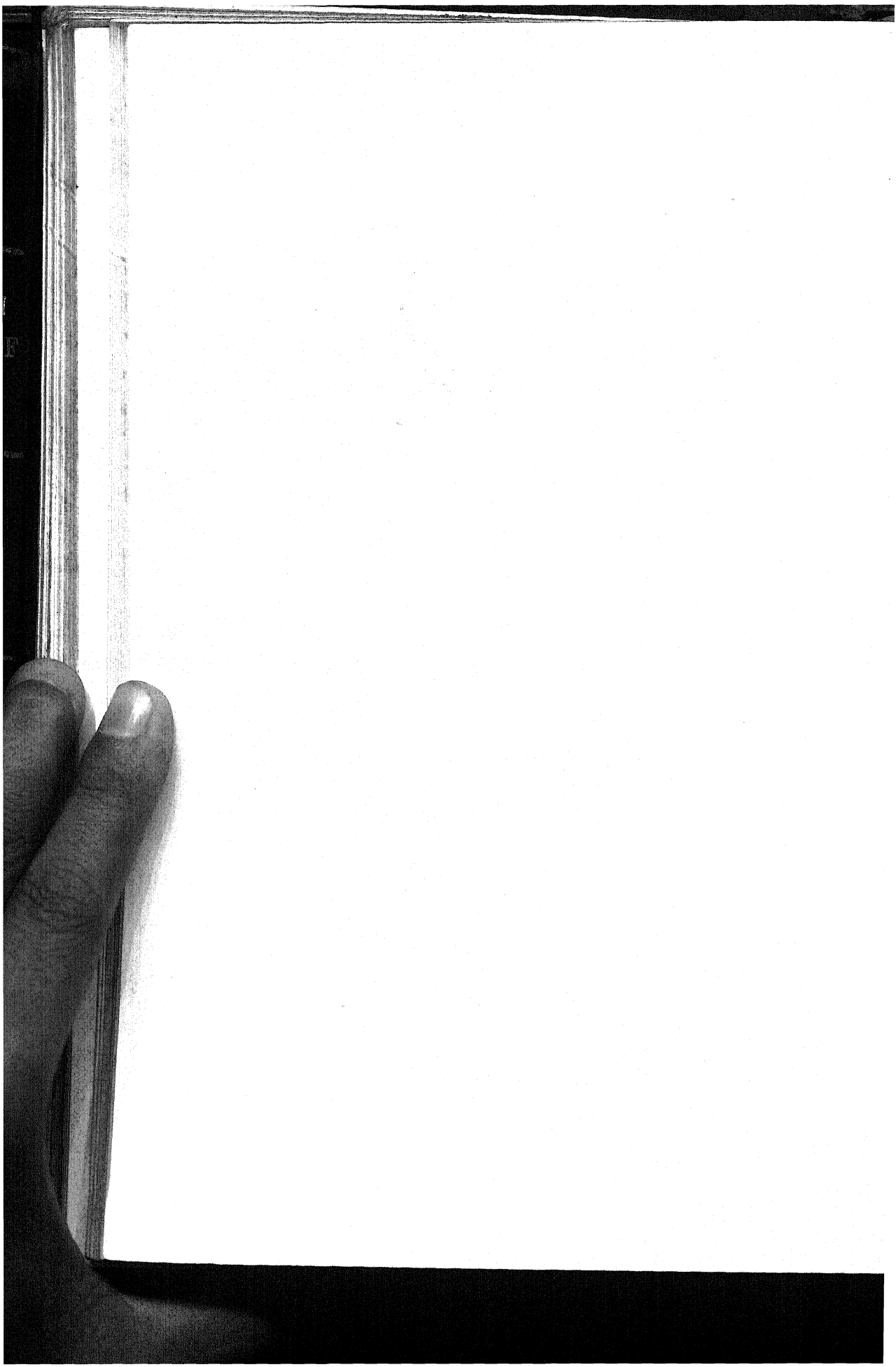
191. *T. lateritia* Lev. Common and usually in large masses on logs of many kinds from Feb. to July in the Willamette valley.

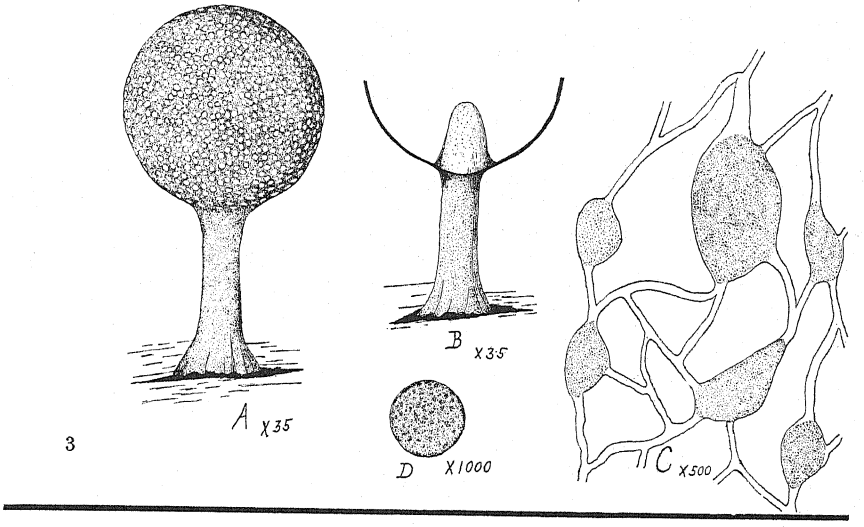
192. *Oligonema brevifilum* Peck. One specimen, found on the under side of a log in a wet area in April in the Willamette valley.





PECK AND GILBERT: MYXOMYCETES





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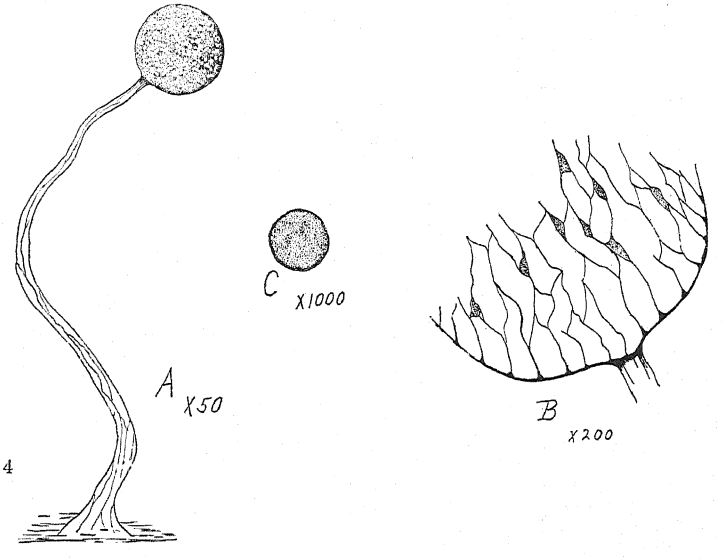

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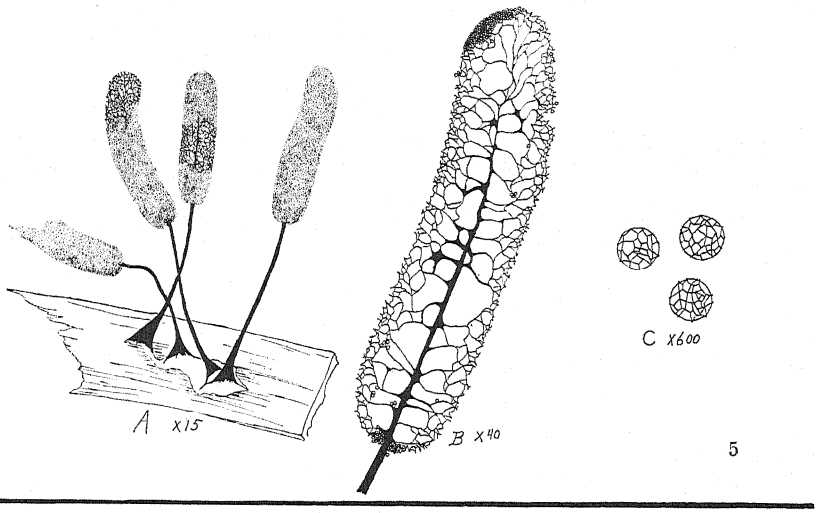
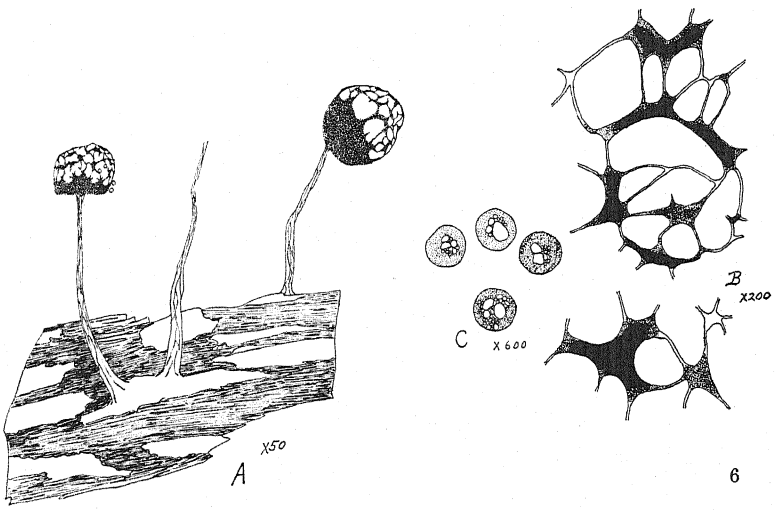
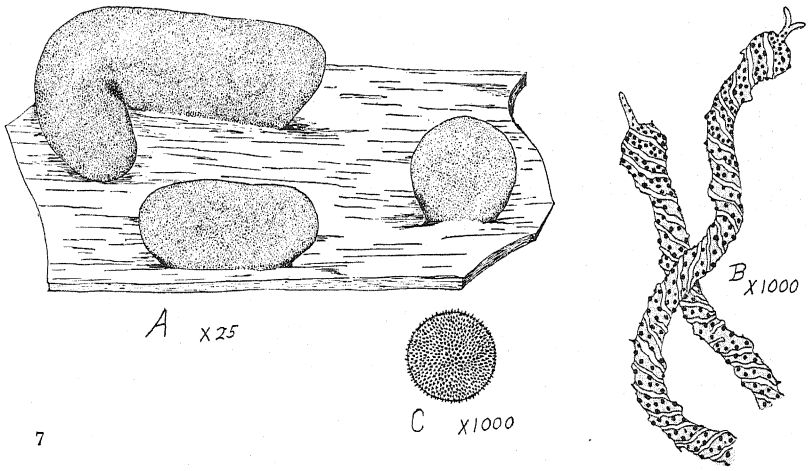
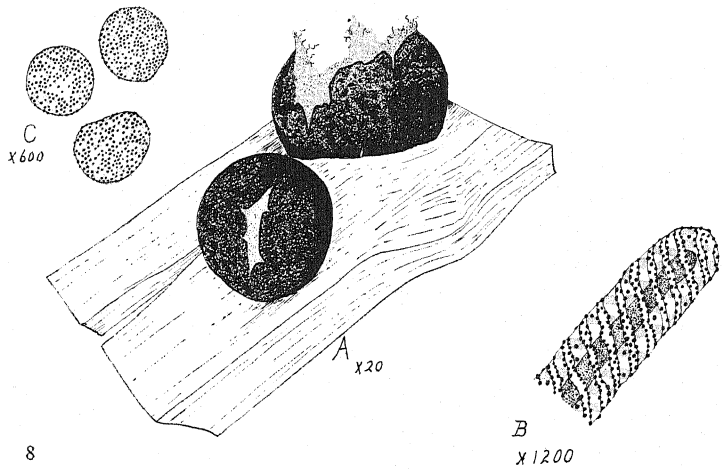
PECK AND GILBERT: MYXOMYCETES





PECK AND GILBERT: MYXOMYCETES





PECK AND GILBERT: MYXOMYCETES

193. *O. flavidum* (Peck) Mass. Rare, on the under side of ash and alder logs in wet areas in Nov. and Dec. in the Willamette valley.

194. *O. nitens* (Lib.) Rost. Rare, in the soil under an old brush pile in March in the Willamette valley.

The authors wish to express their thanks to Dr. T. H. Macbride and to Dr. G. W. Martin, of the University of Iowa, for assistance, particularly in the determination of some of their specimens.

Descriptions of the figures in Plates X–XIII are to be found in the text.

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THE ABSORPTION OF WATER BY ROOT SYSTEMS OF PLANTS [1]

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INTRODUCTION

Botanists are generally agreed upon the manner in which water moves after it has entered the conducting vessels of the plant, but they are by no means agreed upon the manner in which it passes from the soil to the conducting vessels of the roots. Water may move by mass flow for hundreds of feet through the xylem vessels or tracheids, drawn in a state of tension by the removal of water from the xylem vessels of the leaves during transpiration. However, in passing from the soil to the xylem vessels of the root it must cross a mass of living cells, a fact which introduces problems not encountered in its movement through the cavities of the dead vessels or tracheids of the xylem.

In approaching this problem it should be remembered that there usually are important differences between the conditions found in the xylem vessels of actively transpiring plants and those found when little or no transpiration is occurring. In actively transpiring plants the water in the xylem vessels is usually under tension, i.e. exhibits negative pressure. When transpiration is slow and the soil contains an abundance of moisture, as in the spring of the year, the water in the conducting vessels often exhibits a positive pressure, resulting in bleeding from cut or broken stems. This positive pressure is referred to in the literature by such terms as "root pressure," "sap pressure," and "exudation pressure."

In view of these facts it seems very probable that the complex of factors bringing about the absorption of water by rapidly transpiring plants may be quite different from that bringing about the absorption of water when little transpiration is occurring.

From the earliest days of plant physiology, workers have taxed their ingenuity to explain the absorption of water and the development of "root pressure" in plants, but few of the early theories were based on actual experimental work. The earliest workers were unable to give any very definite explanations of the processes involved, though capillarity was supposed to play an important part in the absorption of water and solutes.

In the 19th century more definite theories began to appear. DeCandolle (1832) applied the term "spongioles" to the absorbing regions of the roots. He believed the spongioles were actually contractile and, aided by capillarity, sucked in water from the soil. Dutrochet (1837), who is generally credited

[1] Papers from the Department of Botany, The Ohio State University, No. 277.

with the discovery of the process of osmosis, said the absorption of water and the development of "root pressure" were osmotic phenomena, but he did not attempt to explain exactly how either process occurred.

Sachs (1887) pointed out that a positive pressure or "root pressure" is only developed when there is little transpiration. He believed that under such a condition the osmotic pressure developed in the cells adjacent to the xylem vessels of the root might force water from them into the vessels. However, he was unable to explain why water is forced through only the inner walls of these cells into the vessels and not through the outer walls away from them. He thought that when transpiration is rapid the cavities of the vessels are filled with air, and water then moves through the cell walls. He believed that the loss of water from the leaves by transpiration sets up a pull in this imbibed water which would be continuous through the water-filled cell walls from the xylem to the surface of the roots, causing an inward flow of water from the soil. It has since been shown that the amount of water moving through the walls of the vessels is totally inadequate to replace that lost in transpiration. However, according to the present theory of the ascent of sap, the movement is due to forces set in action by the evaporation of water from the mesophyll cells of the leaf.

Dixon (1910) suggested that the state of tension existing in the conducting vessels of a rapidly transpiring plant may be continuous across the cortex of the root to the menisci in the external walls of the epidermal cells. The water removed from the walls by such a pull is replaced by water from the soil. The rôle of the cells of the root in such an absorption process is a passive one and the root might be compared to a filter.

Atkins (1916) believed that an appreciable concentration of sugar is maintained in the conducting vessels of the roots by "secretion" from the adjoining parenchyma cells. As a result the vessels contain a solution with an osmotic value high enough to cause an inward movement of water by osmosis from the soil through a differentially permeable membrane composed of the living cortical cells of the root. As long as the cells of the cortical tissue remain turgid the fact that their osmotic pressure is much higher than that of the sap in the vessels would not prevent osmosis across this tissue. Dixon and Atkins (see Atkins, 1916) studied the concentration of the sap in the vessels of the roots and stems of several deciduous trees and found it to be highest near the time of opening of the buds in the spring. A high concentration at this time would cause an increased inflow of water, producing a hydrostatic pressure which might be responsible for the "root pressure" and bleeding from cut stems characteristic of many plants in the spring. Atkins believed that the positive pressure thus developed brought many of the air bubbles contained in the vessels into solution, reopening channels for the passage of the transpiration stream.

According to Priestley (1920, 1922) water moves along an osmotic gradient from the root hairs to the vascular cylinder of the root. The

vascular cylinder is enclosed in the rigid endodermis which prevents expansion of the cells lying within. Due to the presence of suberized strips the radial walls of the endodermal cells are impermeable to water. For this reason water can pass into the central cylinder only through the tangential walls and the protoplasts and cannot leak out through the radial walls. Priestley believes that the osmotic pressure developed in the closely confined parenchyma cells may become high enough to force water into the vessels.

Blackman (1921) pointed out that an osmotic gradient from root hairs to central cylinder is unnecessary. A gradient of "absorbing power" with the innermost cells having the highest power would suffice and might exist in connection with a reversed gradient of osmotic pressure.

The "force" referred to by Blackman as the "absorbing power" of the cell is termed by Ursprung and Blum (1916), and by Ursprung (1926) the "suction force" of the cell, by Stiles (1922) "suction pressure," and by Beck (1928) "suction tension." All of these terms, in the last analysis, refer to the same physical quantity, the pressure with which water tends to enter the cell.

It was long assumed that water moved from cell to cell along a gradient determined by the relative osmotic values of the cells involved. The more recent work mentioned above has shown that this is not necessarily the case. Instead, the movement of water from cell to cell is determined by the relative suction tensions of the cells concerned.

Ursprung and Blum (1925) reported the occurrence of regular suction tension gradients in which the suction tension increases from base to apex of the stem and also increases with the lateral distance from the xylem. An exception to this was found in the roots of *Vicia Faba* where the suction tension increases from periphery to endodermis and then decreases. Ursprung (1926) claimed that a difference exists in the suction tension on the inner and outer sides of the endodermal cells which results in the movement of water across the endodermis into the xylem vessels. However, as Beck (1928) has pointed out, it is difficult to understand how a difference in suction tension can be permanently maintained on opposite sides of a cell. Furthermore, it seems questionable whether or not the existence of such a condition has been satisfactorily demonstrated.

Of the so-called vital theories which depend directly on the activity of living cells to explain the absorption of water and the ascent of sap, that of Bose (1927) is best known. Bose claims that "the absorbing root-cells are continuously stimulated by mechanical friction against the soil, giving rise to peristaltic waves of pulsation along the active propulsive layer of the inner cortex." He states that the rhythmic pulsations of these cortical cells are not only responsible for the absorption of water but also force it up the stem. Attempts of other workers (Dixon, 1924) to demonstrate such pulsations have been unsuccessful and even if they do exist they are not necessarily connected with the movement of water.

Livingston (1927), on the other hand, considers that the absorption of water is governed by purely physical forces. He points out that the cell walls are saturated with imbibed water, resulting in a continuous system of liquid water which extends throughout the plant. This hydrostatic system is continuous through the cortical cells, the epidermal cells, and the root hairs with the water in the soil. Removal of water from any part of the plant, by transpiration or other processes, reduces the hydrostatic pressure within the plant and if rapid enough sets up a negative pressure or tension, resulting in an inflow of water from the soil. In Livingston's opinion, the importance of osmosis and internal secretion in the absorption and movement of water is negligible.

Bakhuyzen (1930) has recently suggested that differences in the electrical charges on the inner and outer sides of the endodermis may be responsible for the movement of water and solutes into the xylem and the development of "root pressure." If such differences in charges exist their magnitude would be quite small and it seems probable that their influence on the movement of materials would be correspondingly small.

It is evident that the theories bearing on the absorption of water vary widely in the importance attached to the activity of the living cells of the root. However, they seem to fall into two general groups. In the first group of theories, conditions maintained through the activities of the living cells of the root play the important part in the absorption of water, while in the second group the physical forces concerned operate independently of the activities of the living cells of the root.

The experimental work discussed in this paper was performed in an attempt to throw further light on the actual role of the living cells of the roots in the absorption of water and in the development of positive pressures.

Experimental Work

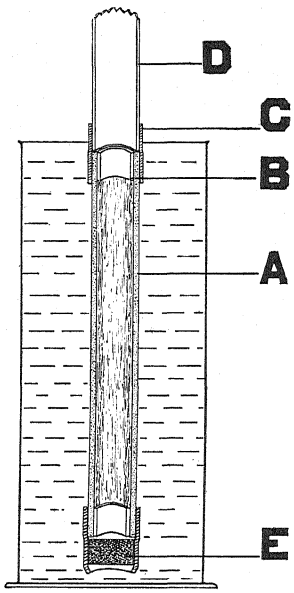
Part I. A Comparison of the Volumes of Water Passing through Plant Tissue by Osmosis with the Volumes of Water Passing through the Same Kind of Tissue when Pressure and Suction Were Applied

Since osmosis plays an important rôle in a number of theories of the absorption of water it seemed advisable to make a comparison of the relative effectiveness of osmosis as contrasted with such physical forces as suction and pressure in the movement of water through plant tissues. The membranes used for these experiments were secured by cutting segments from the hollow petioles of the leaves of the tropical pawpaw, *Carica papaya*, growing in the greenhouse.

The petioles of this plant are often 50 to 60 centimeters in length and a centimeter or more in diameter. A cavity six to eight millimeters in diameter extends through them. The osmotic value of the expressed sap of the petioles, as determined by the cryoscopic method, averaged about nine

atmospheres. The tissue of these petioles is quite compactly arranged and a well developed endodermis is present. Tests with various dyes indicated that there are no openings through which water might enter without actually passing through the cells.

The method of setting up the petiole segments as membranes is shown in text figure 1. The petioles were cut into segments 12 to 14 centimeters in



TEXT FIG. 1. Cross-section through petiole segment set up as an osmometer.

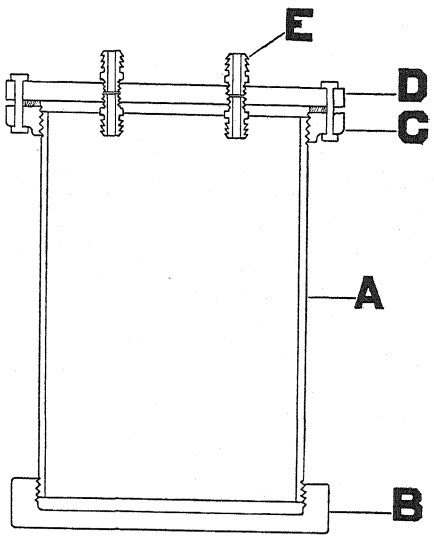
length (*A*). Short pieces of glass tubing (*B*) were slipped into each end of the section to prevent its collapse. Pieces of rubber tubing (*C*) were slipped over each end. The upper end was attached to a glass tube (*D*) while the lower end was closed by a cork stopper (*E*). The connections were all made water tight by an application of shellac and were wrapped with rubber bands as a further precaution against leakage. When completed this made a very satisfactory living membrane of about 50 layers of cells in thickness for use in comparing the rate of water movement by osmosis alone with the rate under pressure and suction.

The structure of the petiole segments employed in these experiments differs somewhat from that of the root. However, it is believed that the differences are not such as would prevent the application of certain conclusions based on results secured with petiole sections to the movement of water through the tissue of the root.

Experiment 1. Measurements of the Volumes of Water Passing through Membranes Composed of Living Plant Tissue by Osmosis.

For the experiments on osmosis the cavities of the membranes were

filled with sucrose solutions of various concentrations. Care was taken to see that no air bubbles were imprisoned during the process. The membranes were then immersed in distilled water and the rate of osmosis determined by measuring the rise of the liquid in the attached tubes. The



TEXT FIG. 2. Cross-section of pressure chamber.

averages of the results are given in table 1 and are also shown as a curve (curve *A*) in text figure 3.

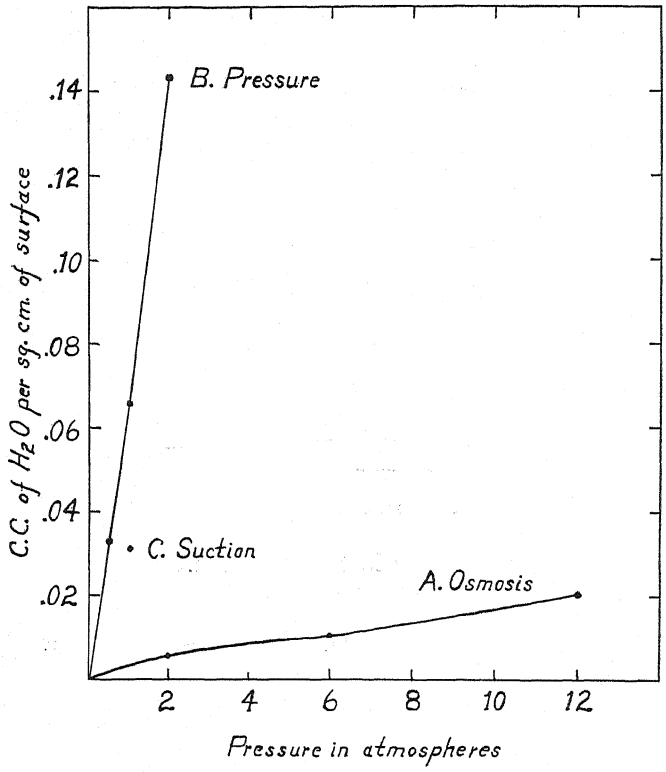
TABLE 1. *Average Volumes of Water Entering Petiole Osmometers Filled with Sucrose Solutions of Different Concentrations*

Osmotic Value of Solution in Osmometer	Vol. of H_2O Entering per sq. cm. of Area in 24 Hours	Relative Rates of Movement with Increased Concentration
2 Atm.	0.0055 cc.	1.0
6 "	0.0107 cc.	1.9
12 "	0.0202 cc.	3.6

Although the osmotic value of the contents of the cells comprising the membranes averaged 9 atmospheres measurable quantities of water passed through the membranes in all experiments where the osmotic value of the solution placed in the cavity of the osmometer was equal to or greater than one atmosphere. As shown in table 1, trebling the concentration of the sucrose solution in the osmometers increased the rate of osmosis only 1.9 times and increasing the concentration 6 times increased the rate of osmosis only 3.6 times.

Experiment 2. Measurements of the Volumes of Water Passing through Membranes Composed of Living Plant Tissue when Mechanical Pressure and Suction Were Applied.

A series of membranes was set up in exactly the same manner as in Experiment 1, except that their cavities were filled with clean quartz sand to prevent the collapse of the walls. The sand was saturated with water and the membranes immersed in distilled water and the air exhausted from within them by means of a vacuum pump attached to the glass tubes. A vacuum of 10 to 20 millimeters of mercury was maintained in the tubes. The



TEXT FIG. 3. Graphic representation of volumes of water passing through membranes by osmosis and under pressure and suction.

amount of water passing in through the walls was determined by measuring the rise of the water level in the attached tubes. An average of 0.0310 cubic centimeters per square centimeter of surface passed through the membranes in 24 hours.

The effects of pressure from without on water movement through the membranes were investigated by means of the pressure chamber shown in text figure 2. The body was made from a piece of 8-inch pipe (*A*), with a cap (*B*), serving as a base. A flange union (*C*) was fitted to the top and a flat cover (*D*) attached to it by means of bolts. Four holes were drilled through the cover and hose connections (*E*) inserted from top and bottom

so that rubber tubing could be attached on both sides of the cover. Membranes were prepared from petiole segments as for the suction experiments, except that they were not attached to glass tubes. Instead they were attached to the hose connections on the lower side of the cover in such a manner that when the cover was in place they would be immersed in the water which partially filled the chamber. Three membranes could be attached at one time, the fourth hose connection being used to attach a pump. Pressures of 7.5, 15, and 30 pounds per square inch above atmospheric pressure were employed. The pressures were produced by means of a tire pump connected with the air space left above the water in the pressure chamber. Glass tubes were attached to the hose connections on the upper side of the cover in order to measure the flow of water into the petiole sections when pressure was applied. The results are given in table 2 and are also shown as curve *B* in text figure 3 where the volume secured by the application of suction is represented by point *C*.

TABLE 2. *Average Volumes of Water Passing through Membranes under Pressure*

Pressure	Vol. of H_2O Passing through per sq. cm. of Area in 24 Hours	Relative Rates of Movement
7.5 lbs.	0.0328 cc.	1.0
15.0 "	0.0660 cc.	2.0
30.0 "	0.1429 cc.	4.3

Discussion

In Experiment 1 increasing the concentration of the sucrose solution in the osmometers always resulted in an increase in the rate of movement of water through the membranes. However, the increase in the rate of movement was not proportional to the increase in concentration. Reference to table 1 shows that trebling the concentration of the sucrose solution increased the rate of osmosis only 1.9 times, while increasing the concentration six times increased the rate of osmosis only 3.6 times. Water passed in through the membranes in all experiments where a sucrose solution having an osmotic value of one atmosphere, or more, was placed in the cavity of the osmometer. When distilled water was placed on both sides of the membrane there was no evidence of movement in either direction. Apparently the movement of water across the membrane by osmosis was not seriously affected by the osmotic value of the contents of the cells comprising the membrane even when that value was higher than the osmotic value of the solution within the osmometer. Evidently water will pass by osmosis across a multicellular membrane into a solution having a lower osmotic value than that of the cells of the membrane, provided that the cells of the membrane are turgid and therefore have a low suction tension. It seems probable that such a movement may occur in roots, water moving from the soil to the conducting vessels across a membrane formed by the turgid cortical cells. These results agree with those expected on the basis of the suction tension theory of the movement of water through plant tissues.

A comparison of the figures for suction and pressure given in text figure 3 shows that over twice as much water per unit of area passed through the membrane in a given period of time when a pressure of one atmosphere or 15 pounds per square inch was applied to the outside as passed through when the pressure was reduced to 10 to 20 millimeters of mercury on the inside though the difference between the pressure inside and outside was approximately the same for both suction and pressure. However, the magnitudes of the pressures involved were different. When the air was exhausted by a vacuum pump the pressure on the water inside the membrane was reduced to almost zero while the pressure on the water outside the membrane still amounted to one atmosphere. When a pressure of one atmosphere was applied to the water outside the membrane the total pressure on the water outside amounted to two atmospheres while there was a pressure of one atmosphere on the water inside the membrane. It is suggested that under suction the air in the tissue may expand so that air bubbles are trapped in the intercellular spaces, reducing the rate of movement of water. Under pressure such bubbles might contract and be displaced by water, resulting in an increased flow.

As long as either pressure or suction was applied to the membranes, bubbles appeared in the attached tubes. These probably consisted chiefly of dissolved air which came out of solution due to the reduced pressure inside of the membrane. The bubbles probably also contained some CO_2 formed during respiration by the living cells of the membrane.

It appears that the rate of movement of water by osmosis across a multicellular membrane composed of living cells is very slow as compared to its movement under mechanical pressure. It seems doubtful whether or not sufficient water could enter the roots of an actively transpiring plant by such an osmotic process as the one described to replace that lost in transpiration.

Part II. The Rôle of the Living Cells of the Roots in the Absorption of Water by Actively Transpiring Plants

As stated in the introduction, various workers have suggested that when a plant is transpiring rapidly enough to set up a tension in the water conducting vessels this tension may be continuous from the xylem across the cortex of the root to the walls of the epidermal cells and root hairs. The removal of water from the xylem vessels would result in the withdrawal of water and a lowering of the saturation of the absorbing cells of the root and consequently an inflow of water from the soil would then occur.

If this is true, an increase in the rate of transpiration should increase the tension in the hydrostatic system and consequently increase the amount of water absorbed, provided that the roots were in moist soil. The living cells of the roots would play but little part in such a process other than to act as absorbing surfaces. A series of experiments was planned to test these suggestions in which the conditions occurring in the water conducting vessels

during transpiration were partially duplicated by attaching a vacuum pump to the cut end of the stem of the plant.

Experiment 1. A Comparison of the Volumes of Water Exuding from the Cut Stems of Plants with and without the Application of Suction.

Several species of potted plants having well developed root systems were used in this experiment. The pot and lower part of the stem were submerged in water and the stem cut off below the surface of the water. A glass tube was then attached to the stump by means of a piece of rubber tubing. The pot was removed from the water and the connections made air and water tight by an application of sealing compound. The amount of water exuding due to "root pressure" was determined for a twelve hour period by measuring the rise of the water column in the attached tube. The air was then exhausted from the tube by means of a vacuum pump and the amount of water secured under reduced pressure ascertained for a twelve hour period. The results are summarized in table 3, the figures given for each species being averages of several plants.

TABLE 3. *A Comparison of the Volumes of Water Exuding from Cut Stems of Various Species of Plants Due to "Root Pressure" Alone, and under Suction*

	"Root Pressure"	Suction	Percent Increase Secured by Adding Suction
Bryophyllum calycinum	0.12 cc.	0.74 cc.	516%
Coleus blumei	0.16 cc.	1.08 cc.	575
Fuchsia magellanica	0.50 cc.	9.66 cc.	544
Helianthus annuus	1.12 cc.	2.84 cc.	153
Pelargonium hortorum	0.38 cc.	1.96 cc.	415
Picea excelsa	0.00 cc.	0.00 cc.	—
Lycopersicum esculentum	0.92 cc.	4.93 cc.	435

The amount of water exuding from the cut stems due to "root pressure" alone was increased by 153 to 575 percent for the same period of time when suction was applied. Apparently only a slight difference between the pressure inside and outside the plant results in a marked increase in the rate of movement of water into and through the root system. No water exuded from the cut stems of the Norway spruce seedlings even when suction was applied.

Experiment 2. A Comparison of the Volumes of Water Secured by Applying Suction to Living Root Systems with the Volumes Secured by Applying Suction to the Same Root Systems after They had been Killed.

The root systems used in Experiment 1 were killed by immersing the pots in hot water until a soil temperature of 58 to 60° C. was attained near the center of the pot. A vacuum pump was attached to the dead root systems and the amount of water secured in a twelve hour period was measured and compared with the amount secured from the same root systems while they were alive. The soil surrounding both living and dead root systems

was saturated during the time suction was applied. The data given in table 4 are averages for several individuals of each species.

TABLE 4. *A Comparison of the Volumes of Water Passing through Living and Dead Root Systems under Suction*

	Living Roots	Dead Roots	Percent Increase Secured by Killing Root Systems
Fuchsia magellanica	9.66 cc.	17.80 cc.	84%
Helianthus annuus	3.57 cc.	67.50 cc.	1790
Pelargonium hortorum	1.48 cc.	4.99 cc.	237
Phaseolus vulgaris	1.90 cc.	5.00 cc.	163
Picea excelsa	0.00 cc.	1.85 cc.	—
Lycopersicum esculentum	3.15 cc.	13.64 cc.	333

Evidently killing the cells of the roots decreased the resistance to water movement, as more water was secured in all cases from the dead root systems than had been obtained from the same root systems while alive.

Experiment 3. Observations on the Behavior of Transpiring Plants after the Roots Were Killed.

Since water so readily passed through dead root systems when suction was applied to the cut stems, experiments were performed to determine whether or not the removal of water by transpiration would be equally effective in causing an intake of water from the soil. If this were the case the tops of transpiring plants should remain alive and unwilted although the roots were killed.

The roots of a number of tomato plants were killed in the manner described in Experiment 2. This method of killing left the tops uninjured. These plants were kept well watered and their behavior was compared with that of plants with living root systems which were growing in soil to which no water was added. If the plants with dead roots were able to secure water from the moist soil they should remain unwilted after the plants with living roots had absorbed the available water from the unwatered soil and had wilted. Similar experiments were performed with sunflower and bean plants. In no case did the plants with dead roots wilt as soon as the plants with living roots which were growing in similar unwatered soil.

The roots of a number of sunflower seedlings were killed by heating the water in which they were growing to 58° C. The water in which the roots were immersed was changed daily and the plants remained unwilted for 7 to 10 days. Evidently the plants were able to absorb water for a time even though their roots were entirely dead. All the plants were kept on a table in the greenhouse, exposed to sunlight, during the course of these experiments.

Discussion

The results of these experiments agree in most respects with those secured by other workers. Jost (1916) likewise found that the application of suction to the stumps of plants gave varying results. The flow of water was

considerably increased in plants which normally exhibit such an exudation from cut surfaces, but only small quantities were secured from plants which do not normally show exudation without suction. In no case was the amount of water obtained sufficient to meet the requirements of even moderate transpiration. The amount of water secured was not proportional to the amount of suction applied, the amount of water increasing more rapidly than the amount of suction. Bode (1923) found that the amount of water secured by applying suction to *Phaseolus* root systems was directly proportional to the amount of suction employed. He also found that under constant suction the amount of water secured increased with increased temperature. No work on dead root systems was reported.

Reference to table 3 shows that reducing the pressure at the surface of the cut stems to approximately zero always markedly increased the movement of water into and through the root system. In rapidly transpiring plants negative pressures of many atmospheres often occur in the hydrostatic system. It appears that the absorption of water might be proportionately increased in such cases by the much greater difference in pressure inside and outside the plant, provided that the roots were in moist soil where the available water supply did not limit absorption.

Such a theory reduces the rôle of the living cells of the roots to that of a mere absorbing surface, a rôle which might in some respects be filled as well by dead as by living cells. This view is supported by the fact that more water always passed through when suction was applied to the dead roots than when suction was applied to the same root systems while alive (see table 4). Furthermore, experiments with tomato, bean, and sunflower plants indicate that sufficient water can be absorbed through dead root systems to replace that lost in transpiration and used in other processes.

The plants lived one to two weeks after their roots had been killed. It appears probable that their eventual death was not entirely due to desiccation, but at least in part resulted from the escape of deleterious substances from the dead cells of the roots. Dixon (1914) noted that when a portion of a stem is killed by heat the leaves above the killed portion soon die. His investigations indicated that death is in part due to the effects of toxic substances escaping from the dead cells in the killed portion of the stem and in part to the clogging of the vessels by gum-like substances. The clogging of the vessels by bacteria might also be a factor in the death of the plants in the present experiments. No data were obtained on the rate of decomposition of the dead root systems.

The three-year-old seedlings of *Picea excelsa* showed a marked difference in behavior from the other plants investigated. Not only was there no evidence of the development of positive pressures in the roots, but no water was secured when suction was applied to the living root systems. Considerable quantities of water were obtained (see table 4) when suction was applied to the dead root systems. Some of the spruce seedlings were placed

in the pressure chamber and a pressure of 12 to 15 pounds per square inch applied. Water was forced through the root systems into the attached tubes. This is in agreement with the results secured in Part I, Experiment 2, where one atmosphere of pressure on the outside was found to be more effective than a vacuum on the inside in causing the movement of water through plant tissue.

The increased volume of water secured from dead root systems as compared with living root systems may perhaps be attributed to the fact that the protoplasts of the living cells offer considerable resistance to the movement of water and this is lessened by the changes in viscosity and permeability brought about by the death of the cells. The water obtained from the dead root systems was yellow in color, probably due to the presence of organic matter which escaped from the dead cells.

The results of these experiments seem to justify the conclusion that in actively transpiring plants the living cells of the roots play no direct part in the absorption of water beyond the maintenance of widely distributed absorbing surfaces. Indirectly they are important in that they prevent the entrance of air into the conducting vessels and by growth continually extend the absorbing area of the roots.

It appears that the movement of water from the soil into the conducting vessels of actively transpiring plants may be due to the tension or negative pressure existing in the hydrostatic system of the plants. The removal of water from the conducting vessels tends to reduce the pressure inside the plant below the pressure exerted on the water in the soil. The difference in pressure may be less than one atmosphere or if a tension is developed in the conducting vessels it may amount to many atmospheres.

The movement of water under such conditions has been termed a mass flow. However, it has been objected that if it is a mass flow minerals would enter with the water, and the work of Hasselbring (1914), Muenscher (1922), and others shows that the absorption of minerals proceeds independently of the absorption of water. It has been called an osmotic process, but the movement is not due to a difference in concentration of solutes on opposite sides of a differentially permeable membrane. The decreasing gradient of vapor pressure (or increasing gradient of suction tension) from the periphery to the vessels of the root is largely due to the decreased pressure in the water-conducting vessels and not to the presence of a higher concentration of solutes in the vessels. Whether the movement of water across the cortex of the root is termed a mass movement or a diffusional process depends principally on the definition of the term employed. Whatever terminology may be preferred, it appears that the cells of the root have the rôle of a passive filter in the process.

Part III. The Rôle of the Living Cells of the Root in the Development of "Root Pressure"

The explanation given in Part II of the absorption of water by actively transpiring plants could not apply to plants in which a positive pressure exists in the conducting vessels. The numerous theories advanced to explain the development of positive pressures all depend in some manner on the presence of living cells in the roots. In the present investigation no exudation of water from cut stems was observed after the roots were killed. If the presence of living cells is necessary, as this fact seems to indicate, the problem is to determine their rôle in the process.

It was long supposed that there was an osmotic gradient from the periphery to the central cylinder of the root, the root hairs having the lowest osmotic pressure and the cells adjacent to the xylem having the highest osmotic pressure. However, this did not explain how water passes from the parenchyma cells with a high osmotic pressure into the vessels which are known to contain a solution of much lower osmotic pressure. Various mechanisms have been proposed which might "secrete" water into the vessels but none of them have been shown to be actually operative.

According to another view the living cells of the root act as a complex, differentially permeable membrane across which water moves by osmosis from the soil to the dilute solution in the xylem vessels. The same principle was involved in the operation of the osmometers used in experiments described in Part I, Experiment 1. It was found in that experiment that the movement of water across a multicellular membrane of living tissue was apparently not seriously affected by the osmotic value of the contents of the cells comprising the membrane, even when that value was higher than the osmotic value of the solution within the osmometer. Similarly, so long as the cells of the cortical tissue of the root remain turgid the fact that their osmotic value is much higher than that of the sap of the vessels would not prevent the movement of water from the soil to the vessels by osmosis.

If such a mechanism actually operates in roots no "root pressure" should be exhibited by plants the roots of which are immersed in a solution with an osmotic value as high as or higher than that of the sap in the xylem vessels.

Experiment 1. The Effects of Immersing the Root in Sucrose Solutions on the Development of Positive Pressure.

A number of sunflower seedlings were grown to a height of 8 to 10 inches in tap water. When the tops were removed and glass tubes attached to the cut stems the presence of positive pressures was indicated by the rise of the water columns in the attached tubes. The water surrounding the roots was replaced by a sucrose solution having an osmotic value of one atmosphere, and no further exudation of water occurred. When the roots were returned to water a positive pressure was again manifested. Sunflower seedlings with intact tops remained unwilted in a sucrose solution having an osmotic

value of two atmospheres even when placed under conditions favoring rapid transpiration.

Well rooted chrysanthemum cuttings growing in tap water showed a strong positive pressure when their tops were removed. The exudation of water was greatly reduced when the roots were placed in a sucrose solution having an osmotic value of one atmosphere and an actual loss of water occurred when the roots were immersed in a sucrose solution having an osmotic value of two atmospheres.

Discussion

It appears that in plants in which little transpiration is occurring and in which positive pressures are manifested, most of the water absorbed may move from the soil to the conducting vessels by osmosis across the differentially permeable membrane formed by the living cortex of the root.

This theory is supported by the fact that immersing the root systems in a solution having an osmotic pressure of one or two atmospheres prevented the development of positive pressure and the accompanying exudation of water from the cut stems. The actual concentration of the sap contained in the vessels of the roots of these plants was not determined. However, it seems probable that the concentration was lower, or at least no higher, than the concentration of the sucrose solutions which stopped the exudation of water.

According to Atkins (1916) the higher concentration of sugar in the vessels in the spring causes an increased inflow of water by osmosis. This results in an increased hydrostatic pressure and the appearance of the bleeding phenomena characteristic of that season. MacDougal, Overton, and Smith (1929) point out that all sap pressures found in the stems of plants should not be attributed to the osmotic action of the roots as other factors are probably involved. They state that capillary absorption by the vessels and wood cells and hydration of the adjacent tissues may play a part in the development of such pressures.

The fact that sunflower plants with attached tops remain unwilted in sucrose solutions in which the root systems with tops removed showed no exudation of water indicates that water was absorbed by the complete plants under conditions where the decapitated plants absorbed no appreciable quantity or in some cases even lost water. This supports the view that the complex of forces concerned in the absorption of water by plants in which little transpiration is taking place differs from the complex of forces concerned in the absorption of water by an actively transpiring plant.

Summary

An investigation has been carried out to determine the rôle of the living cells of the root in the absorption of water and in the development of "root pressure." A distinction is made between the absorption of water by actively transpiring plants and by plants in which little transpiration is occurring, as the complex of factors bringing about the absorption of water under the two sets of conditions is apparently quite different.

1. A multicellular membrane composed of a number of layers of living cells may act as a differentially permeable membrane. Such a membrane is formed by the cortex of the root.

2. The fact that the osmotic value of the contents of the cells comprising the membrane is considerably higher than the osmotic value of the solution on either side of the membrane does not prevent the movement of water across the membrane by osmosis.

3. It appears that under conditions of reduced transpiration most of the water absorbed by plants may move from the soil to the conducting vessels of the roots by osmosis across the differentially permeable, multicellular membrane formed by the living cortex of the root. The accumulation of water in the vessels may result in the development of a positive hydrostatic pressure or "root pressure."

4. Positive pressures were manifested at the cut surfaces of stems only when the roots were alive. Immersing the living root systems in dilute sucrose solutions stopped the exudation of water from the cut stems.

5. The rate of movement of water by osmosis through a multicellular membrane composed of living plant tissue was found to be much slower than the rate of movement through the same kind of membrane when suction or a pressure of one atmosphere was applied.

6. In view of the slow movement of water by osmosis it is doubtfu whether or not an actively transpiring plant could absorb enough water by the usual type of osmotic process to replace that lost in transpiration.

7. Experiments were performed in which the conditions occurring in the water-conducting vessels during transpiration were partially duplicated by attaching a vacuum pump to the cut stem of the plant. It was always found that the amount of water exuding from the cut stems was much greater in a vacuum than under atmospheric pressure.

8. The results of experiments in which the root systems were killed and suction applied indicate that in moist soils as much (or more) water can be absorbed for a few days through dead root systems as through the same root systems while alive.

9. The tops of plants with dead root systems remained alive and unwilted for several days, indicating that sufficient water was being absorbed through the dead roots to replace that lost in transpiration and used in other processes.

10. The movement of water from the soil into the conducting vessels of the roots of actively transpiring plants is probably largely due to the state of tension set up in the hydrostatic system of the plant when water is removed by transpiration or other processes.

11. The rôle of the living cells of the roots in such an absorption process is apparently a passive one. Indirectly they are important as absorbing surfaces, in preventing the entrance of air into the vessels, and in the extension of the absorbing area by growth.

This work was carried on at the Ohio State University during the years 1929–1931. The writer wishes to express his appreciation to Dr. E. N. Transeau, under whose direction the work was performed, and to other members of the staff who offered helpful suggestions.

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TWO NEW TROPICAL AMERICAN SPECIES OF *ADIANTUM*

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The two new maidenhair ferns here proposed have recently been collected rather commonly in the course of botanical exploration in Panamá and Central America. They were recognized by each of the writers independently as probably undescribed, and are based wholly upon material in the U. S. National Herbarium and the Gray Herbarium of Harvard University.

Adiantum decoratum Maxon & Weatherby, sp. nov.

§ *Euadiantum;* species pulchra rachibus paleis subulato-capillaceis divaricatis persistentibus numerosissimis ubique praeditis insignis, habitu *A. pulverulento* non dissimilis sed ut videtur *A. glaucescenti* affinis, quod differt: rachibus gracillimis lucidissimis paleis carentibus, segmentis tenuiter membranaceis mediocriter lobatis, soris vero lunatis valide discretis partem mediam late retusam loborum insidentibus.

Rhizome short-creeping, 2–5 cm. long, 4–7 mm. thick, coarsely and unevenly nodose from the stout pseudopodia of old fronds, closely paleaceous, especially toward apex; scales tufted, light to dark brown, 4–5 mm. long, reticulate, lance-subulate, deciduously ciliate toward the base, remotely denticulate toward the long-attenuate tip. Fronds 2 or 3, approximate, laxly ascending, 40–90 cm. long; stipes somewhat longer than the blades, stout, atropurpureous, obtusely trigonous, deeply sulcate ventrally, lustrous, conspicuously shaggy with very numerous, wide-spreading, subulate-filiform scales (4–5 mm. long, mostly with appressed fimbriate-stellate base), these extending freely to the rachises throughout; blades impari-bipinnate (rarely 3-pinnate at base), 25–50 cm. long, 20–40 cm. broad; pinnae 7–16 (22), 2–4 cm. broad, linear-oblong to elliptic-oblanceolate, alternate, spreading, simply pinnate or (exceptionally) the 2 or 3 lowermost pairs fully bipinnate at base; segments 15–30 pairs, distinctly petiolulate, non-articulate, oblique, the median ones rhombic-oblong from a subrectangular or broadly cuneate inequilateral base, straight or lightly falcate, rounded-obtuse or (if fertile) often muticous at apex, 1.5–2.5 cm. long, 5–8 mm. broad, the basal ones reduced, short-rhombic to orbicular-flabelliform, the terminal ones lanceolate to narrowly and unequally lobate-hastate; sterile pinnae entire at lower margin, unequally serrulate at upper margin and apex; veins prominulous, rather close, mostly 2 or 3 times forked; leaf tissue membrano-herbaceous, dark green, lustrous, and striolate above, beneath glaucous, often conspicuously so; sori borne mostly on segments of the lower half of the pinnae, oblong or sometimes sublunate, 2–3 mm. long, occupying the upper margin and apex, adjacent, the reflexed marginal lobe very narrow, closely revolute, glabrous, the true indusium ample (0.5–0.8 mm. broad), brownish, membranous, glabrous, erose to coarsely dentate-fimbriate; spores triplanate, obtusely 3-lobed, about 35 μ in diameter, yellowish, granular.

Type in the U. S. National Herbarium, no. 1,216,077, collected along the Río Tapia, province of Panamá, Panamá, in wet forest, Dec. 7, 1923–Jan. 11, 1924, by Paul C. Standley (no. 26216).

The following additional specimens have been examined:

PANAMÁ: Near Tapia River, *Maxon & Harvey* 6721. Between Bella Vista and Las Sabanas, *Killip* 2879. Along Chepo River, *Killip* 2675. Along Río Chilibre, *Piper* 5670. Ancón Hill, *Killip* 2500.

COSTA RICA: Cerro Turubares, Orotina, alt. 250 meters, *Jiménez* 602. Capullín, on the Río Grande de Tárcoles, Prov. Alajuela, alt. 80 meters, *Standley* 40174. Nicoya, alt. 200 meters, *Cook & Doyle* 655. Matambú, Nicoya Peninsula, alt. 600 meters, *Cook & Doyle* 693. Nicoya, *Tonduz* (Herb. Mus. Nac. C. R., no. 13766). Gorge of Machuca River, near San Mateo, alt. 50–300 meters, *Biolley* (Pittier, no. 2017); *Biolley* (Herb. Mus. Nac. C. R., no. 17392). Cangrejal de Aserri, April, 1906, *Biolley* s. n.

NICARAGUA: Region of Braggman's Bluff, *Englesing* 219.

GUATEMALA: Los Amates, alt. 50 meters, *Deam* 488.

HONDURAS: San Pedro Sula, Dept. Santa Bárbara, alt. 300 meters, *J. D. Smith* 5645.

Of the material just cited only two or three specimens had previously been named. In aspect the larger fronds of *A. decoratum* somewhat suggest *A. pulverulentum*, although the prevailingly monosorous segments, the non-glaucous under surfaces, and the subtomentose rachises sharply distinguish that species technically. The relationship, though not close, is rather with *A. glaucescens* Kunze, of the Guianas, which differs greatly in its slender non-paleaceous rachises, delicately membranous leaf tissue, and widely discrete sori, the segments being distinctly lobed and bearing the truly lunate sori in the broadly retuse middle portion of the lobes. The widely divaricate, mostly curved, capillary scales, which thickly clothe the stipe and rachises of *A. decoratum* throughout, are distinctive in this group of species.

Adiantum Killipii Maxon & Weatherby, sp. nov.

§ *Euadiantum;* facie staturaque et numero pinnarum necnon forma et dispositione segmentorum *A. latifolio* simile, sed rhizomate breviter repente nodoso nec funiforme et laevi, segmentis subtus laxe et sparse flexuoso-villosis nec glabris, et lobis fertilibus reflexis hirtellis facillime distinguendum.

Rhizome creeping (5–15 cm.), 3–6 mm. thick, densely nodose from the short pseudopodia of old fronds, paleaceous at exposed apex; scales tufted, bright brown, lustrous, 3–4 mm. long, linear- or lance-attenuate to a filiform tip, distantly retrorsely denticulate. Fronds closely tristichous, ascending, 35–80 cm. long, the stipes usually a little longer than the blades, stout, atrocastaneous, triangular-sulcate, lustrous, beset with pale fibrillose scales, glabrescent; blades 20–35 cm. long, about as broad, impari-bipinnate, the atrocastaneous rachises thickly beset with pale appressed fibrillose scales and dark brown spreading subulate scales with dilated substellate bases; pinnae 3–8, alternate, spreading or laxly ascending, lance-oblong to linear-

oblong or oblong, petiolate (5–10 mm.), the terminal one longest; segments 10–20 pairs, approximate or subdistant, spreading, subsessile, non-articulate, straight or subfalcate, oblong to linear-oblong from a subrectangular or broadly cuneate inequilateral base, rounded-obtuse at apex, 2–4 cm. long, 5–15 mm. broad, the basal ones reduced, rhombic-orbicular, the terminal lanceolate, long-acuminate, inequilateral, free, sometimes shallowly rounded-lobate at one side; sterile margins evenly denticulate-serrate; midrib obscure, developed only in basal third; veins mostly 2 or 3 times forked, elevated above, sparsely beset beneath with long, brown, stiff, simple, curved or flexuous, septate hairs; leaf tissue firmly membrano-herbaceous, pliable, above lustrous, dark green, glabrous, and obliquely "sclerotic"-striate between the veins, beneath glaucous; fertile pinnae predominating; sori occupying the whole margin beyond the extreme base, mostly linear, discrete, straight or arcuate, the reflexed marginal lobules hirtellous, the narrow true indusium glabrous, minutely glandular-erose; spores triplanate, obtusely 3-lobed, about 35 μ in diameter, yellowish, granular.

Type in the U. S. National Herbarium, no. 863,376, collected on Ancón Hill, Canal Zone, in deep woods, Dec. 29, 1917, by E. P. Killip (no. 2752).

Other material examined is as follows:

PANAMÁ: Ancón Hill, C. Z., in moist dense or open woods, *Killip* 2503, 2510, 2587, 12068; *Piper* 5589. Barro Colorado Island, Gatun Lake, *Standley* 31330; *Kenoyer* 53; *Wetmore & Woodworth* 120. Pacora, *Killip* 2725. Juan Diaz, *Killip* 2545. Cuatro Calles, near Matachin, C. Z., *Pittier* 4042. Frijoles, C. Z., *Killip* 2834. Porto Bello, *Billberg*, in 1826.

TRINIDAD: Path from Macqueripe to Diego Martin, alt. 60 meters, *Hombersley* 330.

FRENCH GUIANA: In declivis montium Guyanae centralis, Oyapok superius, *Leprieur*, in June, 1833.

The present species, which was first collected by Billberg in Panamá more than a hundred years ago, is beautifully represented in the series brought together by Mr. Killip. Superficially, detached fronds often bear a close resemblance to those of *A. latifolium;* but as noted above, *A. Killipii* differs obviously from that species in its persistently flexuose-villous under surfaces and in its short-creeping, strongly nodose rhizome and subcespitose fronds. In *A. latifolium* the fronds are borne at wide intervals on the greatly elongate, smooth, slender, cordlike rhizome.

In spite of the disparity in size *A. Killipii* is probably more closely related to *A. terminatum* Kunze, of South America, especially in its hairy pinnae and in rhizome characters; but in that species the segments are several times smaller and are linear-oblong, decurved, more freely flexuose-hirsute beneath, and not at all glaucous, and the sori are very much smaller, frequently minute and suborbicular, then appearing like a very narrow moniliform border.

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SOME PROPERTIES OF PLANT SUBSTANCES IN RELATION TO CLIMATE OF HABITAT—VOLATILE OILS, SAPONINS, CYANOGENETIC GLUCOSIDES, AND CARBOHYDRATES

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The object of this paper is to show the climatic and taxonomic distribution in gymnosperms and angiosperms of volatile oils, saponins, cyanogenetic glucosides, and carbohydrates, as well as the variations in the general physical and chemical properties of these substances in relation to the climates which produce them.

VOLATILE OILS

Data and Definitions

Data as to approximately 938 volatile oils have been assembled and reclassified for this paper from Gildemeister's "The Volatile Oils."

Volatile, essential, or ethereal oils are aromatic volatile substances of an oily nature usually obtained by the distillation of vegetable products with steam. They are generally liquid, though sometimes semi-solid at ordinary temperatures, slightly soluble in water, soluble in alcohol, ether, benzene, light petroleum, and most organic solvents. They are found in all parts of the plant or tree, some occurring in the woody stems or roots, others in the bark, leaves, flowers, and fruits. Many essential oils are complex mixtures, containing constituents belonging to various classes of organic compounds, as hydrocarbons, alcohols including phenols, aldehydes, acids, esters, phenol ethers, ketones, lactones, quinones, oxides, bases, sulfides, mercaptans, nitriles, and isothiocyanates. In some cases the oil consists almost entirely of one constituent, *e.g.*, bitter almond oil (benzaldehyde), black mustard oil (allyliso-thiocyanate), and wintergreen oil (methyl salicylate). They are distinguished from fatty oils by their acrid taste, volatility, aromatic odor, solubility in alcohol, non-greasiness, and non-glycerine content.

Climatic and Taxonomic Distribution

The essential oil-bearing families of the gymnosperms and angiosperms as recently listed by Gildemeister and Wolff and divided climatically according to Engler and Gilg (1919) and Willis (1925) are as follows. Tropical: Pandanaceae, Palmae, Araceae, Zingiberaceae, Urticaceae, Menispermaceae, Anonaceae, Myristicaceae, Monimiaceae, Lauraceae, Pittosporaceae, Erythroxylaceae, Humiriaceae, Zygophyllaceae, Burseraceae, Meliaceae, Anacardiaceae, Sapindaceae, Gonystilaceae, Tiliaceae, Sterculiaceae, Guttiferae, Dipterocarpaceae, Winteraceae, Myrtaceae, Apocynaceae, Asclepiadaceae,

Convolvulaceae, Acanthaceae, Rubiaceae, Piperaceae, Turneraceae, Caricaceae, Vitaceae, Orchidaceae, Moraceae, Araliaceae, and Verbenaceae; tropical-subtropical: Tropaeolaceae, Theaceae, Geraniaceae, Iridaceae, and Magnoliaceae; subtropical: Hamamelidaceae and Myricaceae; subtropical-temperate: Taxaceae; temperate: Salicaceae, Juglandaceae, Betulaceae, Caryophyllaceae, Ranunculaceae, Papaveraceae, Cruciferae, Saxifragaceae, Cistaceae, Umbelliferae, Primulaceae, Gentianaceae, Resedaceae, Caprifoliaceae, Pinaceae, Polygonaceae, and Pyrolaceae; and widely distributed: Gramineae, Ulmaceae, Leguminosae, Polygalaceae, Aquifoliaceae, Celastraceae, Violaceae, Thymeliaceae, Oleaceae, Scrophulariaceae, Compositae, Santalaceae, Aristolochiaceae, Rutaceae, Euphorbiaceae, Lythraceae, Solanaceae, Malvaceae, Liliaceae, Rosaceae, Ericaceae, Labiatae, Valerianaceae, and Chenopodiaceae.

The climatic distribution of essential oil families may be compared with the climatic distribution of the total number of plant families. Making use of the climatic distribution of these as determined from Engler and Gilg, and Willis, it becomes apparent that of the 295 families of gymnosperms and angiosperms 87, or 29.49%, contain essential oils. Of a total number of 153 tropical families 38, or 24.84%, have essential oils; of 15 tropical-subtropical families 5, or 33.33%, have essential oils; of 8 subtropical families 2, or 25.00%, have essential oils; of 7 subtropical-temperate families 1, or 14.28%, has essential oils; of 50 temperate families 17, or 34.00%, have essential oils; and of 57 families of widely distributed habitats 24, or 42.10%, have essential oils.

Of the 87 plant families from which volatile oils have been obtained 38, or 43.65%, are tropical; 5, or 5.74%, are tropical-subtropical; 2, or 2.29%, are subtropical; 1, or 1.14%, is subtropical-temperate; 17, or 19.51%, are temperate; and 24, or 27.58%, are widely distributed.

General Properties

Acid Value. The acid value is a measure of the free acids in the volatile oil. It is the number of milligrams of potassium hydroxide necessary to neutralize the free fatty acids in 1 gm. of oil. Table 1 shows the maximum and average acid values of the temperate oils to be greater than those of the tropical.

Saponification Value. This is the quantity of potassium hydroxide in milligrams necessary to saponify 1 gm. of oil. According to table 1 the maximum and average saponification values of the temperate oils are greater than the corresponding values of the tropical.

Ester Value. This value is the difference between the saponification value and the acid value. It is therefore the number of milligrams of potassium hydroxide needed to saponify the neutral esters in 1 gm. of oil. The ester values (table 1) correspond to those of the acid and saponification values in being larger for the temperate oils than for the tropical. It is

evident, therefore, that the quantity of esters as well as their constituent parts (alcohols and acids) is greater for volatile oils of temperate climates than for tropical oils.

Refractive Index. This is the ratio of the velocity of light in the oil compared with its velocity in air under the same conditions. The maximum, minimum, and average refractive indices for oils of temperate plants are lower than the equivalent values of tropical oils. As high refractive values may denote a small number of double bonds it may be that tropical oils contain a greater number or larger amounts of unsaturated substances than temperate oils. A high refractive index may also indicate a large quantity of compounds of high molecular weight.

Optical Rotation. Optical activity is the power of a substance or solution to rotate the plane of vibration of polarized light either to the left or to the right. It is characteristic of compounds containing an asymmetric atom—usually a carbon atom. Most volatile oils have this property. Temperate oils according to the maximum, minimum, and average figures in table 1 are more laevorotatory than tropical, while tropical are more dextrorotatory. A superabundance of laevorotatory compounds may therefore be expected in tropical oils, while the opposite may be expected in temperate. The deviation is of approximately equal intensity in both cases.

Specific Gravity. This is the ratio of the density of a volatile oil to the density of water determined under the same conditions. The maximum and average specific gravities for temperate oils are higher than for tropical oils, but the minimum is lower for temperate. There is a general relationship between the chemical composition of an oil and its specific gravity. A specific gravity of less than 0.90 indicates the presence of a large amount of terpenes or of compounds of the fatty series. The oil of *Pinus Sabiniana* with $d_{15}^{\circ} =$ 0.70, heracleum oil, with $d_{15}^{\circ} =$ 0.80 to 0.88, and oil of rue with $d_{15}^{\circ} =$ 0.833 to 0.840, belong to those with a very low specific gravity. These consist mainly of derivatives of the fatty series. Oil of orange, with specific gravity 0.848 to 0.857, consists largely of the terpene limonene; turpentine oil, with a specific gravity of 0.850 to 0.875, almost completely of hydrocarbons $C_{10}H_{16}$ of the terpene series. A specific gravity higher than 0.90, as is the case with most oils, indicates a mixture of several classes of compounds; whereas a specific gravity of more than 1.0 indicates the presence of compounds of the aromatic series, or, if the compound contains sulfur or nitrogen, of sulfides, nitriles, or isosulfocyanides. From the consideration of both chemical constitution and climate of habitat it may be inferred (table 1) that terpenes and compounds of the fatty series predominate in tropical oils, while temperate oils contain more aromatic, sulfur, and nitrogen compounds. It has been noticed in the oil of anise (*Pimpinella Anisum* L.) that a low index of refraction carries with it a concomitant increase in specific gravity (Gildemeister). This rule according to the present work seems to apply likewise to the average of all essential oils (table 1).

TABLE 1. *General Chemical and Physical Properties of Volatile Oils in Relation to Climate*

	Min.	Max.	Aver. Min.*	Aver. Max.	True Aver.
Acid Value					
Temperate	0.0	250	12	12	12
Tropical	0.0	77	3	5	4
Saponification Value					
Temperate	0.0	228	110	66	88
Tropical	0.0	192	37	27	32
Ester Value					
Temperate	0.0	288	33	63	48
Tropical	0.0	179	17	40	28
Refractive Index					
Temperate	1.3905	1.5582	1.4842	1.4916	1.4879
Tropical	1.4123	1.606	1.4897	1.4968	1.4932
Optical Rotation					
Temperate	0.0	+365 to −105	+20	+30	
			−17	−27	3
Tropical	0.0	+126 to −280	+13	+25	−4
			−14	−33	
Specific gravity at 15° C.					
Temperate	0.6863	1.188	0.9091	0.9355	0.9232
Tropical	0.823	1.155	0.9125	0.9251	0.9188
Boiling Point					
Temperate	96	310	171	219	195
Tropical	130	300	194	225	209

* Average minimum is obtained by averaging all the lowest values given. Average maximum is obtained by averaging all the highest values given of a series. True average is obtained by dividing the sum of the average minimum and average maximum by 2.

Boiling Point. The values for the boiling point of volatile oils indicate higher boiling temperatures for those oils of tropical origin than for those of temperate origin. Most of these values, however, are quite close.

Alcohols

Alcohols of both the aliphatic and aromatic series are found in volatile oils.

Aliphatic Alcohols. Of the primary alcohols of the saturated or methane series but few representatives have thus far been found in volatile oils. In the free state they are found but rarely and then only the lower homologues occur; they are combined more commonly, however, with fatty acids. The occurrence of both free alcohols and free acid may in most instances be attributed to the saponification or hydrolysis of esters during the process of distillation. If the material to be distilled contains carbohydrates, it may happen that the presence of free alcohols is due to fermentation previous to the distillation. Thus, *e.g.*, ethyl alcohol has been observed in the distillate of rose petals that had remained heaped for a short time only.

Of unsaturated aliphatic alcohols the hexylene alcohol $C_6H_{12}O$ which occurs in the tea leaf oil, and the alcohol $C_8H_{16}O$, probably an octylene alcohol, which occures in Gaultheria oil, may be mentioned as representatives of the lower members of the series. Of much greater interest, however, are the

terpene alcohols $C_{10}H_{18}O$, linalool, geraniol, and nerol, and also the unsaturated chain alcohol $C_{10}H_{20}O$, citronellol. These occur as such and as esters in volatile oils in which they are important constituents because of their fragrance.

The cyclic (aromatic) alcohols include benzyl alcohol, phenyl ethyl alcohol, phenyl propyl alcohol, and cinnamic alcohol. The alicyclic (hydroaromatic) alcohols contain in their number dihydrocuminic alcohol, α-terpineol, terpinenol, dihydrocarveol, menthol, sabinol, borneol, etc. The aliphatic aromatic series are also represented in volatile oils by the sesquiterpene alcohols. The aliphatic sesquiterpene alcohols represented include nerolidol and farnesol. More important than the aliphatic are the cyclic sesquiterpene alcohols. As a rule the amount in which they occur in the oils is smaller than that of the sesquiterpenes, although there are oils, like the East Indian sandalwood oil, that consist almost entirely of sesquiterpene alcohols. Chemically, a distinction is made between primary, secondary, and tertiary sesquiterpene alcohols. From another point of view they may be classified into dicyclic and tricyclic compounds. Among the dicyclic are santalols, amyrols, and betulol, and the tricyclic include cedrol, cubeb camphor, and patchouli alcohol.

The alcohols with a predominantly temperate climatic distribution are n-hexyl, octyl, thymol, carveol, sabinol, dihydrocarveol, isoborneol, fenchyl, tenacetyl, bupleurol, dihydrocuminic, betulol, cedrenol, cedrol, daucol, and androl. The list of predominantly tropical alcohols includes: ethyl, isobutyl, amyl, isoamyl, n-heptyl, anisic, nonyl, carvacrol, eudesmol, myrtenol, olibanol, pinocarveol, borneol, geraniol, linalool, nerol, α-terpineol, uncineol, turpinenol, citronellol, eugenol, iso-eugenol, undecyl, matico camphor, tumerol, farnesol, caparrapiol, cubeb camphor, globulol, maali, and guajol. The alcohols widely distributed as to climate of habitat are: methyl, n-butyl, methyl ethyl propyl, benzyl, phenyl ethyl, ethyl amyl carbinol, cuminic, phenyl propyl, neomenthol, santalol, amyrol, atractylol, gilipol, ledum camphor, nerolidol, patchouly, and cholesterol.

Physical and Chemical Properties. From table 2 it is evident that the maximum, minimum, and average molecular weights, melting points, and boiling points of the tropical alcohols are lower than the corresponding values of the temperate alcohols. As widely distributed climatic habitats include both tropical and temperate zones it might be expected that the substances formed by plants living in these localities might have average properties intermediate between those of the tropical and temperate substances. The maximum and minimum values, however, might be either lower or higher than those of the substances produced in predominantly tropical or temperate habitats.

From a chemical standpoint all classes—saturated and unsaturated aliphatic alcohols, terpene alcohols, aromatic and hydroaromatic alcohols, aliphatic, dicyclic, and tricyclic sesquiterpene alcohols—seem to be widely distributed.

Acids

The list of predominantly tropical acids includes: formic, acetic, n-butyric, n-valeric, n-caproic, benzoic, anthranilic, n-caprylic, cinnamic, pelargonic, cumic, capric, palmitic, oleic, myrrholic, and p-methoxy-cinnamic. Those assigned to the temperate habitat group number three: angelic, hydroxymyristic, and hydroxypentadecylic. The widely distributed acids are: propionic, isobutyric, isovaleric, methyl ethyl acetic, oenanthylic, isooenanthylic, salicylic, lauric, stearic, methacrylic, succinic, citronellic, teresantalic, phenylacetic, and veratric.

Physical and Chemical Properties. In table 2 it is seen that the maximum molecular weights, and the maximum and average melting points and boiling points, are higher for tropical than for temperate acids. The other figures, namely the minimum and average molecular weights and the minimum melting points and boiling points, indicate higher values for temperate than for tropical acids. As the number of temperate acids found is very small they may not give truly representative values for this group.

Esters

Esters occur in most volatile oils and are frequently of essential importance so far as the fragrance of these oils is concerned. Because most of them have pleasant odors they play an important rôle in perfumery. As illustrations of oils rich in esters, the oils of bergamot, lavender, geranium, Siberian pine needle, and Roman chamomile may be mentioned. It rarely happens that an oil consists almost exclusively of esters. Such is the case, however, with the distillates of *Gaultheria procumbens* and *Betula lenta*, which consist to the extent of about 99% of methyl salicylate, and which are commonly known as wintergreen oil.

The list of determined tropical esters furnishes: amyl acetate, methyl benzoate, amyl propionate, benzyl acetate, methyl salicylate, hexyl propionate, styrolyl acetate, phenyl propyl acetate, terpinyl formate, amyl butyrate, cinnamyl acetate, terpinyl acetate, ethyl p-methoxy cinnamate, amyl eudesmate, linalyl isobutyrate, terpinyl valerate, and geranyl valerate. The esters of temperate distribution are: ethyl butyrate, hexyl acetate, octyl acetate, methyl methoxy salicylate, octyl n-butyrate, octyl-l-capronate, and octyl caprinate. Those of widely distributed climatic habitats number: ethyl valerate, methyl anthranilate, methyl cinnamate, hexyl butyrate, bornyl formate, methyl n-heptyl carbinol acetate, myrcenyl acetate, bornyl acetate, geranyl acetate, linalyl acetate, methyl acetate, citronellyl acetate, benzyl benzoate, methyl n-nonyl carbinol acetate, linalyl butyrate, bornyl butyrate, citronellyl butyrate, benzyl cinnamate, bornyl isovalerate, bornyl valerate, linalyl valerate, menthyl valerate, menthyl isovalerate, kessyl acetate, cinnamyl cinnamate, and linalyl capronate.

Physical and Chemical Properties. The summary of data in table 2 shows the minimum molecular weights, melting points, and boiling points higher for

TABLE 2. *Physical and Chemical Constants of Volatile Oil Constituents in Relation to Climate of Habitat*

Habitat	Melting Point			Boiling Point			Molecular Weight			Empirical Formula								
										Carbon			Hydrogen			Oxygen		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
									Hydrocarbons									
Tropical	79	−70	−13	273	155	210	236	128	171	18	10	12	24	8	19	—	—	—
Temperate	105	105	105	295	142	242	204	122	174	15	9	13	24	10	18	—	—	—
Widely Distributed	50	−73	−11	275	160	204	204	134	156	15	10	11	24	14	18	—	—	—
									Alcohols									
Tropical	210	−117	21	294	78	205	222	46	157	15	2	10	26	2	17	2	1	1
Temperate	210	−52	53	290	156	217	256	102	167	15	6	10	26	14	19	2	1	1
Widely Distributed	148	−98	19	360	64	231	386	32	171	27	1	11	46	4	19	1	1	1
									Acids									
Tropical	171	−45	71	300	100	214	306	46	155	18	1	8	34	1	14	5	2	2
Temperate	84	45	60	185	185	185	258	100	200	15	5	11	30	8	22	3	2	2
Widely Distributed	185	−80	14	383	141	214	284	74	140	18	3	7	36	6	12	4	2	2
									Esters									
Tropical	50	−75	−24	223	147	192	242	130	180	28	7	11	30	8	16	3	1	2
Temperate	49	−93	27	210	121	175	284	116	190	18	6	11	36	10	22	4	2	2
Widely Distributed	44	−91	9	323	135	233	372	130	215	26	7	13	28	9	20	3	2	2

tropical than for temperate esters. The maximum and average melting points and boiling points of the tropical esters are likewise higher than those of the temperate zone. The average molecular weights and melting points are higher for temperate esters than for tropical. The maximum molecular weight is also in favor of the temperate esters.

The data on esters lead to rather mixed results and consequently the relationships may not be reliable as criteria for the group. However, esters are influenced by the chemical and physical properties of the substances from which they are formed. As those combining alcohols and acids, as shown previously in this paper, have melting points, molecular weights, etc., which vary in accordance with the climates in which they are formed, esters, therefore, may be expected to vary similarly.

Hydrocarbons

Aliphatic Hydrocarbons. The lowest member of the primary (methane or paraffin) series of hydrocarbons C_nH_{2n+2} which has been found in volatile oils is the n-heptane C_7H_{16}, with a boiling point of 98° C.

The higher members of the paraffin, and probably of the olefin (C_nH_{2n} or ethylene) series also appear to be quite widely distributed in the vegetable kingdom. They constitute the wax-like coating and secretion on leaves, flowers, and fruits. In volatile oils, however, they are not commonly met with because of their very low volatility. Sometimes they separate in crystalline form when the oil is exposed to a low temperature or they remain behind upon fractional distillation. Apparently these hydrocarbons seldom occur alone, but in mixtures of homologues as has been shown in the case of rose oil. Their melting points seldom if ever agree with those of known members of the series. As their climatic and taxonomic distribution has already been studied (McNair 1931) it will not be further discussed here.

Paraffin or olefin hydrocarbons have been found more particularly in the oils distilled from flowers. Of olefinic hydrocarbons, only the octylene, C_8H_{16}, melting point 123° C., has been found thus far, viz., in the oils of bergamot and lemon, but possibly it also occurs in lignaloe oil. Isoprene, C_5H_8, interesting because of its relation to the terpenes, has been observed only as a decomposition product of caoutchouc and turpentine oil.

However, chain hydrocarbons of the formula of saturation C_nH_{2n-4} with three double bonds have been found. In composition they agree with the terpenes but differ in having a lower specific gravity and a lower index of refraction. These hydrocarbons have been termed "olefinic terpenes" by Semmler.

Aromatic hydrocarbons include sequoine, naphthalene, styrene, and cymene.

Alicyclic Hydrocarbons. The principal hydrocarbons occurring in volatile oils are alicyclic. The composition of by far the greater number is represented by the formula $C_{10}H_{16}$; hence they belong to that class of the terpenes

in which the carbon atoms are arranged in a cycle. This group includes santene, pinene, limonine, dipentene, etc.

Sesquiterpenes. In addition to the terpenes $C_{10}H_{16}$, the higher boiling fractions of many volatile oils, viz., those between 250° and 280° C. contain hydrocarbons corresponding to the formula $C_{15}H_{24}$ which have been named sesquiterpenes. A considerable number of these have been isolated, although but few have been investigated.

A few oils consist almost exclusively of sesquiterpenes. In most instances, however, they play but a secondary rôle. Their occurrence is so general that they must be regarded as belonging to the most common constituents of the volatile oils. Based on their molecular refraction and relative density, they are classified as aliphatic, monocyclic, dicyclic, and tricyclic sesquiterpenes. As with the terpenes, these groups are characterized by regularity with reference to boiling point and more particularly as to density.

Hydrocarbons predominantly tropical in origin are: myrcene, naphthalene, α-pinene, fenchene, limonene, dipentene, phellandrene, bisabolene, zingiberene, cadinene, caryophyllene, caparrapene, guajene, heerabalene, and heveene. Hydrocarbons which are found for the most part in temperate plant families are: sequoine, santene, selinene, and cedrene. Plant families of widely distributed climatic habitats include as their hydrocarbons ocimene, cymene, sabinene, camphene, terpinolene, sylvestrene, terpinene, citronella, humulene, and santalene.

Physical and Chemical Properties. The maximum and minimum molecular weights and the minimum boiling points are greater for tropical than for temperate hydrocarbons (table 2). The average molecular weights, melting points, and boiling points are lower for tropical than for temperate hydrocarbons. The maximum and minimum melting points and maximum boiling points are likewise lower for tropical than for temperate hydrocarbons. However, the number of melting points and the number of temperate hydrocarbons (4) may be too small to give representative figures. From a chemical standpoint both the aliphatic and aromatic hydrocarbons seem to be widely distributed climatically; the alicyclic group are slightly more tropical. Of the sesquiterpenes, the aliphatic are widely distributed, the monocyclic are tropical, and the bicyclic more tropical than widely distributed.

Discussion

The hydrocarbons of volatile oils have approximately one-half the molecular weights and carbon and hydrogen content of the wax hydrocarbons (McNair 1931). The quantities for these values increase from tropical to temperate in wax hydrocarbons, while the corresponding figures for volatile oil hydrocarbons are mixed. This uncertainty of values for volatile oil hydrocarbons is probably the result of too few data for all melting points and all temperate values.

The comparatively large amount of data available for alcohols of both

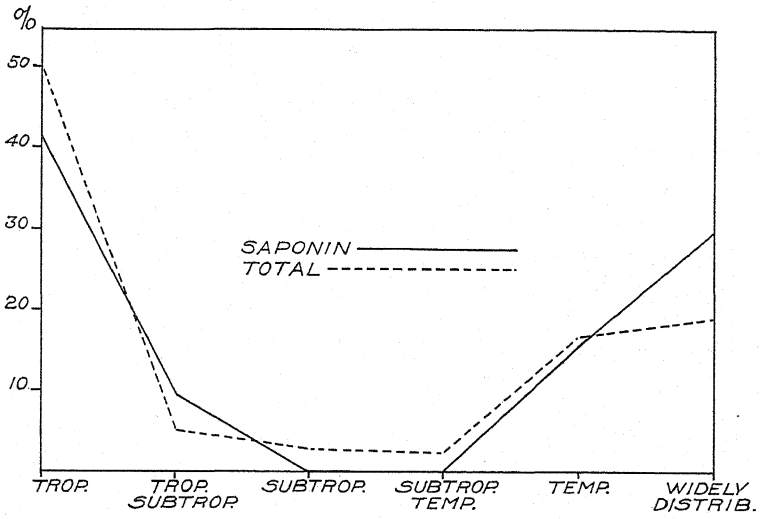
waxes and volatile oils makes for more uniform agreement of results and more positive conclusions. In both instances it seems safe to conclude that the lower values apply to the alcohols of tropical sources rather than to those of the temperate zone.

The acids of waxes likewise have much higher molecular weights than do those of the volatile oils. In both the tendency is apparent to higher values in temperate than in tropical acids.

Saponins

Definition

Saponins include a large number of amorphous glucoside-like bodies of widespread occurrence in the vegetable kingdom and have in common certain chemical, physical, and especially physiological properties. Their aqueous solutions, when shaken, foam rapidly and readily. In this respect they resemble soap. Many saponin substances have a sharp, harsh taste. In powdered form they excite violent sneezing and also irritate the eyes. They are capable of holding many finely divided substances in a state of emulsion. They dialyze incompletely and salts precipitate them from solution. The



Text Fig. 1. The climatic distribution of all saponin-containing plant families compared with all plant families.

saponins all have the three chemical elements carbon, hydrogen, and oxygen. Most saponins are neutral and only a few are faintly acid. Neutral saponins and alkali salts of acid saponin substances dissolve in water and hot aqueous alcohol but are insoluble in absolute alcohol and ether. Saponins occur in roots (*Senega*, *Saponaria*), tubers (*Cyclamen*), barks (*Quillaja*, *Guaiacum*), fruits (*Sapindus*, *Saponaria*), seeds (*Aesculus*, *Agrostemma*,

Thea), stems (*Dulcamara*), and leaves (*Guaiacum*). In fact almost any part of the plant may contain saponins.

Climatic and Taxonomic Distribution of Saponins

According to Kofler and Howes saponins are found in 70 plant families. These families are classified climatically by Engler and Gilg (1919) and Willis (1925). An analysis of their data shows that 30, or 40.3%, are mostly tropical; 7, or 10.0%, are mostly tropical-subtropical; none is mostly subtropical or subtropical-temperate; 12, or 17.1%, are temperate; and 21, or 30.0%, are widely distributed in habitat. The climatic distribution of saponin families may be compared with the distribution of the total number of plant families (text fig. 1). Making use again of the climatic distribution of these by Engler and Gilg, and Willis, it becomes apparent that of the 295 families of gymnosperms and angiosperms 71, or 24.06%, have saponins. Of a total number of 153 tropical families 30, or 42.3%, have saponins: of 15 tropical-subtropical families 7, or 9.9%, have saponins; of 8 subtropical families none has saponins; of 7 subtropical-temperate families none has saponins; of 50 temperate families 12, or 16.1%, have saponins; and of 57 families of widely distributed habitats 21, or 29.6%, have saponins. It is evident that of the plant families producing saponins twice as many are found in the tropics as in the temperate zone. The ratio of tropical saponin families to the total tropical families (19.6%), however, is not much less than the ratio of temperate saponin families to the total temperate families (24.0%).

The 30 tropical saponin families are: Aizoaceae, Apocynaceae, Araceae, Araliaceae, Asclepiadaceae, Bignoniaceae, Combretaceae, Cucurbitaceae, Dioscoreaceae, Guttiferae, Lecythidaceae, Loganiaceae, Loranthaceae, Menispermaceae, Moraceae, Myristicaceae, Myrsinaceae, Myrtaceae, Orchidaceae, Palmae, Phytolaccaceae, Piperaceae, Pittosporaceae, Proteaceae, Rubiaceae, Sapindaceae, Sapotaceae, Urticaceae, Verbenaceae, and Zygophyllaceae. The 7 tropical-subtropical saponin families are: Gnetaceae, Amaryllidaceae, Theaceae, Amarantaceae, Elaeocarpaceae, Iridaceae, and Magnoliaceae. No saponin-producing family that is largely of subtropical or of subtropical-temperate distribution is known. The 12 saponin families that are mostly temperate in distribution are: Berberidaceae, Betulaceae, Cactaceae, Caprifoliaceae, Caryophyllaceae, Gentianaceae, Polemoniaceae, Polygonaceae, Primulaceae, Ranunculaceae, Saururaceae, and Umbelliferae. The saponin families of widely distributed habitats, of which there are 21, are: Chenopodiaceae, Compositae, Dipsacaceae, Euphorbiaceae, Gramineae, Hippocastanaceae, Labiatae, Leguminosae, Liliaceae, Linaceae, Oleaceae, Polygalaceae, Portulacaceae, Rhamnaceae, Rosaceae, Rutaceae, Scrophulariaceae, Solanaceae, Styracaceae, Thymeliaceae, and Violaceae.

Physical and Chemical Constants in Relation to Climatic Distribution

Table 3 brings out certain facts in regard to a probable relationship between climate of habitat and molecular weight, and also between the number of carbon, hydrogen, and oxygen atoms in saponins.

TABLE 3. *Variation in Molecular Weight and Chemical Composition of Saponins in Relation to Climate of Habitat*

Habitat	Molecular Weight			Carbon *			Hydrogen *			Oxygen *		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Tropical	890	374	504	42	17	24	66	26	40	20	9	11
Tropical-subtropical	1293	404	848	60	18	39	92	28	60	30	10	20
Temperate	1168	344	572	66	17	28	104	28	44	17	7	12
Widely distributed	1203	376	589	55	16	28	94	24	45	28	10	13

* Number of atoms.

Molecular Weight. As the molecular weights of only two substances are known that are produced by tropical-subtropical families they may not represent an average analysis of the saponins of this group and will consequently not be given consideration here.

The maximum and average molecular weights for the tropical saponins are lower than the temperate saponins but the minimum molecular weight of tropical saponins is slightly higher than the temperate. Those of widely distributed habitats should form a rough average between those of the tropical and the temperate habitats, as their distribution includes both tropical and temperate climates.

Carbon and Hydrogen Content. The tropical saponins have lower maximum, minimum, and average numbers of carbon and hydrogen atoms per molecule than the saponins of temperate climates. The widely distributed saponins, as might be expected, occupy roughly an intermediate position in this respect.

Oxygen Content. Tropical saponins have a greater number of oxygen atoms per molecule in their largest and smallest molecules than do those of temperate saponins. The average numbers of oxygen atoms per saponin molecule may be more truly representative of the entire group. These figures show the tropical as greater than the temperate. The widely distributed saponins have larger numbers in all three (maximum, minimum, and average) results.

Toxicity in Relation to Climate

In table 4 are found the lethal doses of some saponins administered to mice (Kofler, page 204). These appear in this table associated with their respective saponins and climates of habitat. In this way an approximation of the toxicity of some of the saponins can be determined according to cli-

TABLE 4. *The Relation of Climate of Formation to the Toxicity of Saponins to Mice*

Climate of Formation	Family	Saponin or Its Source	Lethal Dose in Mg. per 1 gm. Mouse	
			Intravenous	Oral
Widely distributed...	Scrophulariaceae	Digitonin	0.010	0.09
Temperate.....	Primulaceae	*Primula*	0.015	0.20
Temperate.....	Caryophyllaceae	*Gypsophila*	0.015	2.00
Tropical.....	Sapindaceae	Sapotoxin	0.020	1.00
Tropical.....	Sapindaceae	Saponin	0.020	6.00
Widely distributed...	Polygalaceae	Senegrin	0.045	1.00
Tropical.....	Sapindaceae	Saponin pur. albiss	0.060	4.00
Tropical.....	Sapindaceae	Saponin powd.	0.100	1.50
Widely distributed...	Hippocastanaceae	*Aesculus*	0.900	3.00

mate. From these figures the lowest lethal doses decrease from tropical to temperate. The doses of saponins of widely distributed habitats appear as the lowest, highest, and intermediate figures. This is a logical dispersal as widely distributed habitats include tropical, temperate, and intermediate climates. Corresponding results are obtained with lethal concentrations of saponins for trypanosomes (Kofler, after Wada, page 115).

It may be concluded from this, therefore, that saponins of tropical origin are less toxic in proportion to weight than saponins of temperate origin. It is interesting to note that the toxicity of alkaloids shows a similar climatic variation (McNair, 1931).

The climatic variation in toxicity is uncertain in the cases of fish poisoning, haemolysis, and the isolated frog's heart, according to the results tabulated by Kofler (1927, pp. 119, 146, and 169–170).

CYANOGENETIC GLUCOSIDES

Definitions

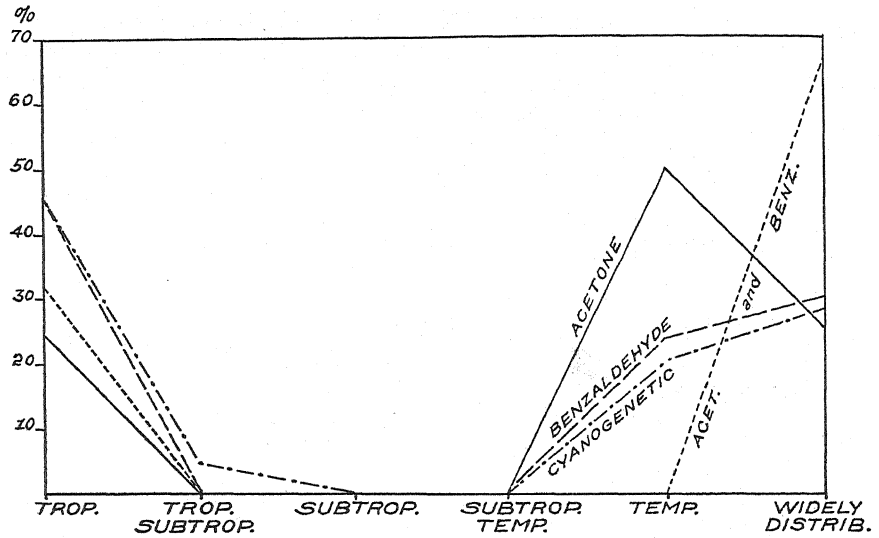
Glucosides are those vegetable products which on treatment with acids or alkalies yield a sugar, or some closely allied carbohydrate, and one or more other substances (frequently phenols or aromatic aldehydes) as decomposition products. Glucosides which yield hydrogen cyanide as one of their products of hydrolysis are known as cyanogenetic or cyanophoric glucosides.

Hydrogen cyanide unites directly with aldehydes and ketones, forming additive products, termed cyanohydrins or hydroxycyanides. This very important reaction $CO + HCN \rightarrow C(OH)(CN)$, which is often used in synthesizing organic compounds, accounts for the union of hydrocyanic acid with aldoses or ketoses. Besides these sugars cyanogenetic glucosides frequently have as hydrolytic products the ketone, acetone, or the aldehyde, benzaldehyde. For example, the glucoside linamarim (phaseolunatin) $C_{10}H_{17}O_6N$ yields on hydrolysis, acetone cyanohydrin and dextrose, while the glucoside

amygdalin $C_{20}H_{27}O_{11}N$ gives hydrocyanic acid, and benzaldehyde, and two molecules of dextrose.

Climatic and Taxonomic Occurrence

According to Greshoff, de Puymaly, Rosenthaler, and Treub, cyanogenetic glucosides are found in 39 plant families of the gymnosperms and angiosperms. These families are classified climatically by Engler and Gilg (1919), and Willis (1925). An analysis of their data (table 5 and text fig. 2)



TEXT FIG. 2. The climatic distribution of cyanogenetic glucosides.

shows that 18, or 46.16%, are mostly tropical; 2, or 5.12%, are mostly tropical-subtropical; none is mostly subtropical-temperate; 8, or 20.51%, are mostly temperate; and 11, or 28.21%, are widely distributed in habitat.

The climatic distribution of cyanogenetic glucoside families may be compared with the climatic distribution of the total number of plant families. Making use again of the climatic distribution of these by Engler and Gilg, and Willis, it becomes apparent that of the 295 families of gymnosperms and angiosperms 39, or 13.22%, contain cyanogenetic glucosides. Of a total number of 153 tropical families 18, or 11.76%, have these glucosides; of 15 tropical-subtropical families 2, or 13.33%, have them; of 8 subtropical families none has them; of 7 subtropical-temperate families none has them; of 50 temperate families 8, or 16.0%, have them; and of 57 families of widely distributed habitats, 11, or 19.3%, have them.

It is evident that of the plant families producing these particular compounds more than twice as many are found in the tropics as in the temperate zone. Those mostly tropical in distribution include 46.16% of all these particular families, while only 20.51% are found growing largely in

TABLE 5. *Climatic Distribution of Families of Cyanogenetic Glucosides*

	Tropical		Tropical-subtropical		Subtropical		Subtropical-temperate		Temperate		Widely Distributed	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cyanogenetic vs. total	18/153	11.76	2/15	13.33	0/8	0	0/7	0	8/50	16.00	11/57	19.30
Cyanogenetic	18	46.16	2	5.12	0	0	0	0	8	20.51	11	28.21
Acetonecyanohydrin	1	25.00	0	0	0	0	0	0	2	50.00	1	25.00
Benzaldehyde cyanohydrin	6	46.15	0	0	0	0	0	0	3	23.07	4	30.77
Acetone and benzaldehyde	1	33.30	0	0	0	0	0	0	0	0	2	66.66

the temperate zone. The ratio of the included tropical families to the total tropical families (11.7%), however, is not quite as great as the ratio of the included temperate families to the total temperate families (16.0%).

The 18 tropical cyanogenetic glucoside families are: Anacardiaceae, Araceae, Asclepiadaceae, Commelinaceae, Convolvulaceae, Flacourtiaceae, Lauraceae, Melastomaceae, Myoporaceae, Olacaceae, Passifloraceae, Proteaceae, Rubiaceae, Sapindaceae, Sapotaceae, Sterculiaceae, Tiliaceae, and Urticaceae. The two tropical-subtropical families in this group are the Canellaceae and the Magnoliaceae. No cyanogenetic glucoside-producing family that is largely of subtropical or of subtropical-temperate distribution is known. The eight families that are mostly temperate in distribution are: Berberidaceae, Caprifoliaceae, Cruciferae, Papaveraceae, Platanaceae, Ranunculaceae, Saxifragaceae, and Scheuchzeriaceae. The cyanogenetic glucoside families of widely distributed habitats, of which there are 11, are: Calycanthaceae, Celastraceae, Compositae, Cornaceae, Droseraceae, Euphorbiaceae, Gramineae, Leguminosae, Linaceae, Rosaceae, and Scrophulariaceae.

Acetonecyanohydrins

The families whose cyanogenetic glucosides are known to yield acetone are: (temperate) Berberidaceae and Ranunculaceae, (tropical) Passifloraceae, and (widely distributed) Linaceae. Those families which contain both acetone and benzaldehyde are: (tropical) Convolvulaceae, and (widely distributed) Euphorbiaceae and Leguminosae. From the above, acetone occurrence is dominant in the temperate zone and, as might be expected, is found with benzaldehyde in families which are of wide climatic distribution.

Benzaldehyde cyanohydrins

The families whose cyanogenetic glucosides are known to yield benzaldehyde are: (temperate) Caprifoliaceae, Cruciferae, and Saxifragaceae; (tropical) Anacardiaceae, Asclepiadaceae, Flacourtiaceae, Rubiaceae, Sapotaceae, and Tiliaceae; and (widely distributed) Compositae, Gramineae, Rosaceae, and Scrophulariaceae. The families that contain both benzaldehyde and acetone have been listed above under the discussion of acetone cyanohydrins. According to the available statistics benzaldehyde is of more common occurrence than acetone in these glucosides and is more tropical than temperate in distribution.

These nitrogenous glucosides contain a constituent of high molecular weight in the tropics, and another of low molecular weight in the temperate zone. That is, benzaldehyde of tropical distribution has a molecular weight of 106, a melting point of $-13°$ C., and a boiling point of 179° C.; while acetone of temperate occurrence has a molecular weight of 58, a melting point of $-94°$ C., and a boiling point of 56° C.

Carbohydrates

Data

The various carbohydrates considered in this paper occur free and combined as glucosides in plants. Such glucoside combinations include saponins, tannins, plant pigments, and polysaccharides. Artificial carbohydrates are excluded. The climatic classification of plant families used is that of Engler and Gilg and Willis.

Pentoses

Arabinose. Arabinose is found in one tropical family, the Araliaceae, and in four families of wide distribution, the Leguminosae, Liliaceae, Rosaceae, and Gramineae. It has been found in a total of seven genera. The climatic occurrences of these genera are: Araliaceae, *Polyscias* (tropical), *Hedera* (temperate); Liliaceae, *Aloe* (subtropical); Leguminosae, *Acacia* (tropical-subtropical), *Vicia* (temperate); Gramineae, *Tridens* (temperate); and Rosaceae, *Prunus* (temperate). According to the habitats of both families and genera, arabinose should have a wide climatic distribution.

Xylose. Xylose is found in the tropical family Bromeliaceae, the temperate family Primulaceae, and the widely distributed families Leguminosae and Gramineae. Its occurrence in genera is: Bromeliaceae, *Puya* (subtropical); Primulaceae, *Primula* (temperate); Leguminosae, *Daviesia* (tropical); and Gramineae, *Avena* (temperate). From its generic and familial distribution it should have a broad climatic occurrence. The fact that it is also found in many woods likewise indicates this.

Ribose. Ribose is known to occur in two families, both cosmopolitan, namely, the Leguminosae and the Gramineae. The two known genera are: Leguminosae, *Lupinus* (widely distributed), and Gramineae, *Saccharum* (tropical). These meager findings indicate for ribose a wide climatic distribution.

Rhamnose. This methyl pentose has been discovered in two tropical families, the Araliaceae and the Anacardiaceae, and in four families of widely distributed habitat, namely, the Leguminosae, Fagaceae, Rhamnaceae, and Rutaceae. The seven genera known to contain it are: Araliaceae, *Hedera* (temperate); Anacardiaceae, *Schinopsis* (subtropical), *Rhus* (subtropical-temperate); Fagaceae, *Quercus* (temperate); Leguminosae, *Robinia* (temperate); Rutaceae, *Barosma* (subtropical); and Rhamnaceae, *Rhamnus* (widely distributed). The range of both genera and families indicates a wide climatic area for rhamnose production.

Rhodeose. Rhodeose or methyl arabinose has been located in a tropical family, the Convolvulaceae, in the tropical genus *Exogonium*.

Hexoses

Mannose. Mannose has been discovered in two tropical families, the Palmae and Orchidaceae; in one temperate family, the Pinaceae; and in one

widely distributed family, the Leguminosae. The known genera are: Palmae, *Phytelephas* (tropical), *Phoenix* (tropical); Orchidaceae, *Orchis* (temperate); Pinaceae, *Picea* (temperate); Leguminosae, *Ceratonia* (subtropical-temperate). It is apparent from the distribution of both families and genera that the climatic region in which mannose is formed covers a wide range.

Glucose. Glucose is found in all those families which contain either starch or saccharose, in both of which it is a component, as well as in families that have such glucosides as saponins, pigments, tannins, etc. Its presence has been detected in the following. Tropical: Moraceae, Anonaceae, Lauraceae, Meliaceae, Anacardiaceae, Sapindaceae, Tiliaceae, Bombacaceae, Guttiferae, Flacourtiaceae, Passifloraceae, Caricaceae, Myrtaceae, Sapotaceae, Ebenaceae, Cucurbitaceae, Araliaceae, Vitaceae, and Rubiaceae; tropical-subtropical: Punicaceae; temperate: Pinaceae, Ranunculaceae, Cruciferae, Saxifragaceae, Umbelliferae, Salicaceae, Caprifoliaceae, and Primulaceae; widely distributed: Fagaceae, Rosaceae, Leguminosae, Euphorbiaceae, Cornaceae, Ericaceae, Labiatae, Compositae, Gramineae, Oleaceae, Rutaceae, Hippocastanaceae, Solanaceae, Liliaceae, Scrophulariaceae, Linaceae, Malvaceae, and Rhamnaceae. From the above it is shown that glucose has been found in 19 tropical, 1 tropical-subtropical, 8 temperate, and 18 widely distributed families. Of the 153 tropical families glucose occurs in 12%, and of the 50 temperate families it has been located in 16%. Glucose is therefore widely distributed.

Laevulose. Laevulose is a component of saccharose but not of starch. Consequently those plant families that contain saccharose will contain laevulose. The laevulose-containing families are as follows. Tropical: Moraceae, Anonaceae, Lauraceae, Meliaceae, Anacardiaceae, Sapindaceae, Bombacaceae, Guttiferae, Flacourtiaceae, Caricaceae, Myrtaceae, Sapotaceae, Ebenaceae and Cucurbitaceae; tropical-subtropical: Punicaceae; temperate: Cruciferae, Saxifragaceae, and Umbelliferae; widely distributed: Rosaceae, Leguminosae, Euphorbiaceae, Cornaceae, Ericaceae, Labiatae, Compositae, Gramineae, Liliaceae, and Oleaceae. Laevulose has been found in 28 families, namely, 14 tropical, 1 tropical-subtropical, 3 temperate, and 10 widely distributed. Of the 153 tropical families it is in 9% and of the 50 temperate families it is in 6%. Laevulose, therefore, is widely distributed.

Galactose. Galactose is found in one tropical family, Bromeliaceae; one temperate family, Caryophyllaceae; and five widely distributed families, Scrophulariaceae, Ericaceae, Rhamnaceae, Malvaceae, and Chenopodiaceae. The various genera represented are as follows: Bromeliaceae, *Puya* (subtropical); Caryophyllaceae, *Gypsophila* (temperate); Scrophulariaceae, *Digitalis* (temperate); Ericaceae, *Vaccinium* (temperate); Rhamnaceae, *Rhamnus* (widely distributed); Malvaceae, *Gossypium* (tropical-subtropical); and Chenopodiaceae, *Beta* (temperate). Based on family distribution,

galactose is widely distributed, but from the arrangement of genera it is dominantly temperate.

Sorbose. Sorbose has so far been found in the temperate genus *Pyrus* of the widely distributed family Rosaceae.

Heptoses

Mannoketoheptose. Mannoketoheptose is found in the tropical genus *Persea* of the tropical family Lauraceae.

Sedoheptose. Sedoheptose is found in the temperate genus *Sedum* of the widely distributed family Crassulaceae.

Disaccharides

Trehalose. Trehalose, a disaccharide formed from two glucose molecules, has been located in the genus *Echinops* in the family Compositae, both widely distributed. It has also been found in the tropical genus *Selaginella* of the tropical family Selaginellaceae.

Vicianose. Vicianose, a glucose arabinoside, is found in the temperate genus *Vicia* of the widely distributed Leguminosae.

Primeverose. Primeverose made from glucose and xylose has been discovered in the temperate genus *Primula* of the temperate Primulaceae.

Strophantobiose. Strophantobiose is a component of the glucoside strophantin. It may be decomposed into mannose, rhamnose, and methyl alcohol and has been found in the widely distributed genus *Strophanthus* of the tropical Apocynaceae.

Saccharose. The disaccharide saccharose is made up of laevulose and glucose. It has been located in the following families. Tropical: Moraceae, Anonaceae, Lauraceae, Meliaceae, Anacardiaceae, Sapindaceae, Vitaceae, Bombacaceae, Guttiferae, Flacourtiaceae, Passifloraceae, Caricaceae, Myrtaceae, Sapotaceae, Loganiaceae, Cucurbitaceae, Palmae, and Bromeliaceae; temperate: Ginkgoaceae, Ranunculaceae, Saxifragaceae, Umbelliferae, and Salicaceae; widely distributed: Fagaceae, Chenopodiaceae, Rosaceae, Rhamnaceae, Cornaceae, Ericaceae, Labiatae, Compositae, Gramineae, Liliaceae, and Polygalaceae. Saccharose has been isolated from 34 families, of which 18 are tropical, 5 are temperate, and 11 are widely distributed. Of the 153 tropical families it is in 11% and of the 50 temperate families it is in 10%. Saccharose consequently may be considered as of cosmopolitan climatic occurrence.

Trisaccharides

Secalose. Secalose (trifructosan), $C_{18}H_{30}O_{15}$, is composed of three molecules of laevulose (fructose) and is found in the Gramineae, a family of wide distribution. Of the Gramineae secalose has been separated from *Lolium* and *Secale*, both temperate in location.

Raffinose. Raffinose, or melitose, $C_{13}H_{32}O_{16} \cdot 5H_2O$, a condensate of galactose, glucose, and fructose, is known to occur in three families, two of

which are widely distributed, namely, the Chenopodiaceae and the Malvaceae; the other family, a tropical one, is the Myrtaceae. The genera known to have raffinose are: Chenopodiaceae, *Beta* (temperate); Malvaceae, *Gossypium* (tropical-subtropical); and Myrtaceae, *Eucalyptus* (tropical). From a consideration of both families and genera raffinose is widely distributed.

Melezitose. Melezitose or melicitose, $C_{18}H_{32}O_6$, is a glucoside of glucose and fructose and so far has been found in two families, one temperate, the Pinaceae, and the other widely distributed, the Leguminosae. The genera in which the substance was discovered are: Pinaceae, *Larix* (temperate) and *Pseudotsuga* (temperate); and Leguminosae, *Alhagi* (subtropical). A consideration of the families indicates a wide distribution, while from the genera a subtropical-temperate habitat is deduced.

Gentianose. Gentianose is a trisaccharide formed from glucose, glucose and fructose contained in the roots of *Gentiana*, a widely distributed genus of the Gentianaceae, a family of wide distribution.

Rhamninose. The trisaccharide rhamninose, with formula $C_{18}H_{34}O_{14}$, is made from galactose and rhamnose. It is obtained from the widely distributed genus *Rhamnus* of the widely distributed Rhamnaceae.

Tetrasaccharides

Stachyose. The tetrasaccharide stachyose, of the formula $C_{24}H_{42}O_{21}$, can be hydrolyzed into fructose, glucose, and galactose. It has been isolated from four families of wide climatic distribution: the Labiatae, Orchidaceae, Oleaceae, and Leguminosae. The containing genera are: Labiatae, *Stachys* (widely distributed); Orchidaceae, *Lamium* (tropical); Oleaceae, *Jasminum* (tropical-subtropical); and Leguminosae, *Phaseolus* (tropical-subtropical) and *Lupinus* (widely distributed). Stachyose, judged from the habitats of its containing families and genera, is widely distributed.

Polysaccharides

Inulin. Inulin, $C_{36}H_{62}O_{31}$ formed from the condensation of laevulose molecules has been discovered in only two families so far, both of which are widely distributed, namely, the Liliaceae and the Compositae. An investigation of the known genera which contain inulin shows that the majority are of temperate habitat, *e.g.*, *Arctium*, *Cynara*, *Taraxacum*, *Balsamorrhiza*, and *Camassia.*

Discussion

The progress in the isolation and identification of carbohydrates in plants is still in its infancy. According to the data at hand of the 295 families of gymnosperms and angiosperms, only 59, or 20.0%, have been analyzed for carbohydrates exclusive of starch. The climatic distribution of habitats as here determined from the meager chemical data available may later be found in error. However, it seems quite likely (table 6) that carbohydrates of temperate climates have a tendency to be of higher molecular weight and to

TABLE 6. *Physical Properties and Climatic Distribution of Carbohydrates*

	Formula	M. W.	M. P.	Heat of Combustion kg. cal. per mol	Climatic Habitat
Arabinose	$C_5H_{10}O_5$	150	160	559	Widely distributed
Ribose	$C_5H_{10}O_5$	150			" "
Xylose	$C_5H_{10}O_5$	150	140	561	" "
Rhamnose	$C_6H_{12}O_5$	164	92	711–718	" "
Rhodeose	$C_6H_{12}O_5$	164			Tropical
Dextrose	$C_6H_{12}O_6$	180	147	673	Widely distributed
Laevulose	$C_6H_{12}O_6$	180	94	675	" "
Mannose	$C_6H_{12}O_6$	180	132		" "
Galactose	$C_6H_{12}O_6$	180	168	670	Temperate or widely distributed
Sorbose	$C_6H_{12}O_6$	180	154	668	Temperate or widely distributed
Mannoketoheptose	$C_7H_{14}O_7$	210			Tropical
Sedoheptose	$C_7H_{14}O_7$	210			Temperate or widely distributed
Vicianose					Temperate or widely distributed
Primeverose	$C_{11}H_{20}O_{10}$	312	210		Temperate
Trehalose	$C_{12}H_{22}O_{11}$	342		1341	Widely distributed
Strophantobiose					" " or tropical
Saccharose	$C_{12}H_{22}O_{11}$	342	160 d.	1349	Widely distributed
Rhamninose	$C_{18}H_{34}O_{14}$	474	137		" "
Secalose	$C_{18}H_{30}O_{15}$	486			Temperate or widely distributed
Raffinose	$C_{18}H_{32}O_{16}$	504	119	2018–2025	Widely distributed
Melezitose	$C_{18}H_{32}O_{16}$	504	149	2042	Subtropical or widely distributed
Gentianose	$C_{18}H_{32}O_{16}$	504	210		Widely distributed
Stachyose	$C_{24}H_{42}O_{21}$	666	169	2709	" "
Inulin	$C_{36}H_{62}O_{31}$	990	160 d.	4130*	Temperate or widely distributed
Starch	$(C_6H_{10}O_5)_x$	$(162.1)_x$		4179*	Widely distributed

* Gram calories per gram.

have higher heats of combustion than those of families of tropical habitat. Such a deduction would be in accordance with the findings for acids, alcohols, esters, and hydrocarbons in essential oils and waxes (McNair 1931).

It has already been determined that some low molecular sugars, the pentoses, because they are found mainly as the principal constituents of plant gums and mucilages, are chiefly tropical in occurrence (McNair 1930). Gums are highly hydrophilic and undoubtedly play a major rôle in the water relations of certain plants, especially the succulent desert plants, such as cacti. This highly hydrophilic property, it should be noted, is not a property of the higher molecular hexose polysaccharides and therefore probably not as important in temperate climates where the higher polysaccharides may be found to dominate.

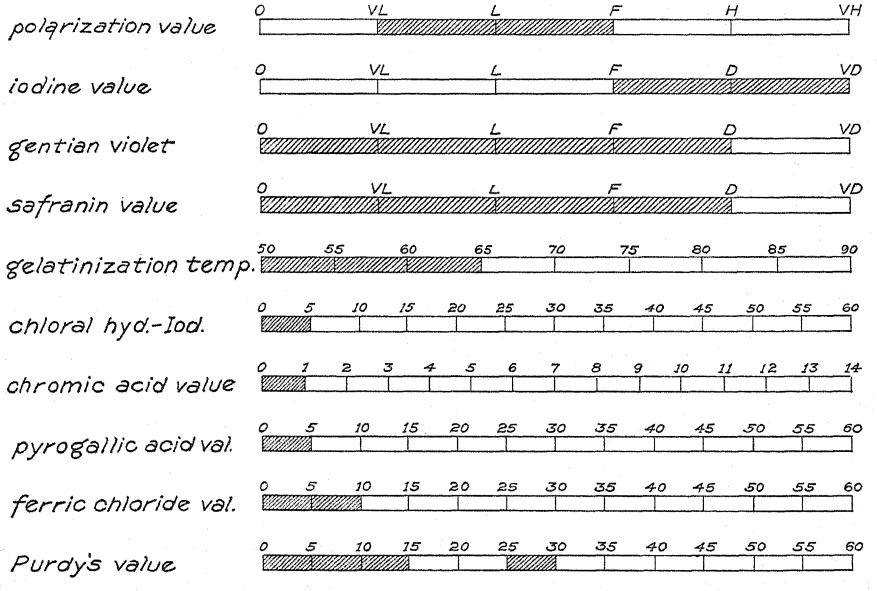
Spoehr (1919) in his experiments with low water content and high temperature found an increase in polysaccharides and pentosans and a decrease in monosaccharides. It may well be, as suggested by Gortner (1929), that

the increase in pentosans was a response of the plant toward the elaboration of hydrophilic colloids to bind the water in the plant against the forces of transpiration and to build up an imbibition pressure to draw water from an already water-depleted soil. Spoehr found that high water content and a low temperature reversed the conditions.

Some Special Properties of Starch in Relation to Climate

No attempt will be made to list all the families that are known to make starch. Those families, however, whose starch has been analyzed by Reichert (1913) are as follows. Tropical: Sapindaceae, Araceae, Marantaceae, Cannaceae, Convallariaceae, Zingiberaceae, Cycadaceae, Gesneriaceae, Convolvulaceae, Musaceae, Taccaceae, and Cucurbitaceae; tropical-subtropical: Amaryllidaceae and Iridaceae; subtropical none; subtropical-temperate: Nymphaeaceae; temperate: Ranunculaceae, Cruciferae, Primulaceae, and Polygonaceae; widely distributed: Gramineae, Liliaceae, Fagaceae, Euphorbiaceae, Solanaceae, and Leguminosae.

Reichert was able to differentiate between 350 different starches from the



TEXT FIG. 3. Some reactions of starches from temperate plant families (shaded portion). *VL*, very low or light; *L*, low or light; *F*, fair; *H*, high; *VH*, very high; *D*, dark; *VD*, very dark. Numbers refer to minutes of reaction time.

above listed families by means of eleven processes of analysis, namely: (1) histological microscopic examination to determine the form and size of the grains, position and character of the hila, characteristics of the lamellae, orientation, etc.; (2) the degree of polarization; (3) the iodine reaction; (4) the gentian violet reaction; (5) the safranin reaction; (6) the temperature of

gelatinization; (7) the chloral hydrate-iodine reaction; (8) the chromic acid reaction; (9) the pyrogallic acid reaction; (10) the ferric chlorid reaction; and (11) the Purdy solution reaction. The results of these reactions classified according to reaction intensity and climatic habitats of the producing families are shown in text figure 3.

It is apparent from these results that starches from temperate families are likely to have (1) a polarization value from very low to high; (2) an iodine color value from fair to very dark; (3) a gentian violet value from zero to dark; (4) a safranin value from zero to dark; (5) a gelatinization temperature from 50° to 90° C.; (6) a chloral hydrate-iodine value from zero to 5 minutes; (7) a chromic acid value from zero to one minute; (8) a pyrogallic acid value from zero to five minutes; (9) a ferric chlorid value from zero to ten minutes; (10) a Purdy's value of from zero to fifteen minutes and from twenty-five to thirty minutes. The starch of *Polygonum* does not always conform with the above, namely in the iodine values, ferric chlorid value, and Purdy's value.

It is evident from the above observations that starches from temperate plant families may be expected to react readily with all chemical reagents listed except the dyes (gentian violet and safranin), to have a wide range of polarization values, and to have higher gelatinization temperatures, and be less "saturated" toward iodine than starch from tropical plant families. Starch from families of widely distributed habitats may have values extending over the entire reaction ranges as these habitats include both temperate and tropical climates. Some starch from other climatic habitats may be found to give some of the reactions of temperate starch.

It is remarkable that the high degree of absorption to iodine by temperate starch is analogous to the high degree of unsaturation of temperate fatty oils, and that the high temperature of gelatinization of temperate starch corresponds to the higher melting points of hydrocarbons, acids, alcohols, esters, and saponins of temperate climates (McNair 1929, 1930, 1931).

SUMMARY

Volatile oils are found in 29% of the angiosperm and gymnosperm families, saponins in 24%, cyanogenetic glucosides in 13%, and carbohydrates in 20% (exclusive of starch). All of these substances (except possibly carbohydrates) are more tropical than temperate in distribution. It seems quite likely that the acids, alcohols, esters, and hydrocarbons of volatile oils, and that saponins and carbohydrates all have higher molecular weights when of temperate origin than when of tropical origin.

Volatile Oils

Volatile oils have been obtained from 87 plant families, or 29% of all the higher plant families. Of these volatile oil-producing families 44% are tropical, 7% are tropical-subtropical, 2% are subtropical, 3% are subtropical-temperate, 18% are temperate, and 25% are widely distributed.

The acid values, saponification values, ester values, refractive indices, optical rotations, specific gravities, and boiling points indicate a variation in value in accordance with the climatic habitat of the source plants. All of these values except the boiling points are lower for tropical oils. It is evident, therefore, that the amounts of esters and their constituent alcohols and acids are greater for volatile oils of temperate climates than for tropical oils.

According to the refractive indices, tropical oils have compounds either of lower molecular weight or of greater unsaturation.

The optical rotation indicates the predominance of dextro compounds in temperate oils and laevo compounds in tropical. The intensity is about equal in both classes.

Volatile oil alcohols, acids, hydrocarbons, and esters also have values which seem to vary with their producing climates.

The molecular weights, melting points, and boiling points have apparently lower values for the tropically produced alcohols, acids, and esters than those of temperate families. The hydrocarbon climatic relationships are uncertain.

The climatic-physical constant ratios of the volatile oil constituents is in accordance with the values of the wax constituents.

The molecular weights of the wax hydrocarbons, alcohols, and acids are nearly double those of the volatile oil hydrocarbons, alcohols, and acids.

Saponins

Saponins are found in 71 plant families; of these 42% are mostly tropical and 16% are temperate. The ratio of tropical saponin families to the total tropical families (20%), is not much less than the ratio of temperate saponin families to the total temperate families (24%).

The maximum and average molecular weights for the tropical saponins are lower than the temperate saponins but the minimum molecular weight of tropical saponins is slightly higher than the temperate.

The numbers of carbon and hydrogen atoms in the saponin molecules increase from tropical to temperate.

The maximum and minimum numbers of oxygen atoms increase and the average numbers slightly decrease from tropical to temperate.

Saponins of tropical origin are less toxic to mice and trypanosomes than those of temperate origin.

Saponins resemble alkaloids in diminution of toxicity from temperate to tropical.

Cyanogenetic Glucosides

Cyanogenetic glucosides are shown to occur in 39, or 13%, of the plant families of the gymnosperms and angiosperms. Of these 46% are tropical, 5% tropical-subtropical, 20% temperate, and 28% widely distributed climatically.

Hydrogen cyanide unites directly with aldehydes and ketones forming

additive products, termed cyanohydrins or hydroxycyanides. This reaction accounts for the union of hydrocyanic acid with aldoses and ketones. Besides these sugars cyanogenetic glucosides frequently have as hydrolytic products acetone or benzaldehyde.

Acetone is shown to occur in the nuclei of these glucosides in four families and is mostly temperate.

Benzaldehyde has been found in the cyanogenetic glucosides of thirteen families which are more frequent in the tropics.

These nitrogenous glucosides contain a constituent of high molecular weight in the tropical zone.

Both acetone and benzaldehyde are found separately in the glucosides of one tropical and two widely distributed families.

Carbohydrates

The progress in the isolation and identification of carbohydrates in plants is still in its infancy. According to the data at hand only 59, or 20%, of the 295 families of gymnosperms and angiosperms have been investigated.

There is an indication that the carbohydrates of low molecular weight, and those with a low heat of combustion and low potential energy are more apt to be found in tropical families than in temperate.

Low molecular weight sugars (pentoses) are the principal constituents of plant gums and mucilages. These are tropical and highly hydrophilic and aid water retention and intake.

Starches from temperate plant families may be expected to react readily with all chemical reagents listed except the dyes (gentian violet and safranin), to have a wide range of polarization values, and to have higher gelatinization temperatures, and be less "saturated" toward iodine than starch from tropical plant families.

Starch from families of widely distributed habitats may have values extending over the entire reaction ranges as these habitats include both temperate and tropical climates.

It is remarkable that the high degree of absorption to iodine by temperate starch is analogous to the high degree of unsaturation of temperate fatty oils, and that the high temperature of gelatinization of temperate starch corresponds to the higher melting points of hydrocarbons, acids, alcohols, esters, and saponins of temperate climates.

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INHERITANCE OF RESISTANCE TO LOOSE AND COVERED SMUT IN A HYBRID OF EARLY GOTHLAND AND VICTOR OATS[1]

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The Early Gothland variety of oats has shown a marked difference in its behavior to the Missouri races of loose (*Ustilago Avenae* (Pers.) Jens.) and covered (*U. levis* (K. & S.) Magn.) smuts. It has proved to be very susceptible to the former and has been almost entirely resistant to the latter. The Victor variety, on the other hand, has been very susceptible to both the loose and covered smuts; in fact it has consistently been one of the most susceptible varieties that I have grown in my various experiments, usually 100 percent of the inoculated plants being infected.

In connection with the experiments with the hybrid recorded in this paper, these two varieties have been inoculated with each of the smuts and grown for direct comparison, the data being recorded in table 1. During the years 1926–1931, 73 plants of Early Gothland and 49 plants of Victor have been inoculated with the loose smut and all of them (100 percent) have been infected. In comparative experiments, 208 plants of Early Gothland have been inoculated with the covered smut and only 2 (slightly less than 1 percent) have been infected, these 2 smutted plants occurring in the field in 1928. Victor, on the other hand, showed high susceptibility, 187 plants out of 188 inoculated (99.4 percent) being smutted. Similar results obtained in many other types of experiments during the same years confirm these results on the behavior of the two varieties with reference to loose and covered smut.

Early Gothland is a late maturing oat with moderately large panicles, the branches of which droop slightly at the outer ends. The spikelets are usually two-grained, rarely three-grained. Awns are rather numerous on the spikelets, but they are not fully developed nor twisted at the base. The glumes of the grains are white or pale yellowish; the outer grains are rather long (18–22 mm.), slender, and long pointed.

Victor is also a late maturing oat. It grows quite tall with large, rank stems and broad leaves. The panicles are large and wide-spreading, with coarse branches drooping from the middle outward. Awns are numerous in the panicle, generally having a twisted base, and frequently geniculate. The spikelets are usually two-grained. The glumes are brownish black to black, although the short tips are nearly white. The grains are large, plump, the outer ones usually about 18–22 mm. long, and short pointed.

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Cross between Early Gothland and Victor

In view of the difference in the behavior of these two varieties towards loose and covered smut, hybrids between them furnish very interesting material for the study of the inheritance of smut resistance. In 1924 a cross was made, Early Gothland being used as the female parent, and the F_1 plant was grown in the greenhouse in 1925. In general, it resembled the Victor parent, the plant being tall, with coarse stems and broad leaves. The panicles were large and loose. Most of the spikelets contained awns, many of which had twisted bases and some were more or less geniculate. The grains were plump like the Victor parent; the glumes were not so dark as those of Victor, being more of a tan or reddish brown color.

During the following years, second and third generation plants of this hybrid have been grown, having been inoculated with either the loose or the covered smut. Brief reports on some of the results obtained have been published (1, 2, 3, 4, 5).

The general methods used in previous investigations have been followed. Inoculated seed was germinated in sand at a constant temperature of about 20° C. Later, the seedlings were transplanted and grown to maturity in the greenhouse or in the field.

Data Obtained with the F_2 Generation

Results with Ustilago Avenae

The data for the second generation are recorded in table 1. In 1926, 52 F_2 plants inoculated with loose smut were planted in the greenhouse and in 1928, 15 additional plants of this generation inoculated with this smut were grown to maturity in the field; thus a total of 67 inoculated F_2 plants of the hybrid were grown, and all proved to be completely susceptible to the loose smut, 100 percent infection having been obtained.

Results with Ustilago levis

The first set of second generation plants inoculated with the covered smut were also planted in the greenhouse in 1926, 44 plants being inoculated, of which 8 (18.1 percent) were infected. In 1928, 50 additional F_2 plants were grown in the field and 2 (4 percent) were infected. Finally, in 1929, 53 inoculated F_2 plants were grown in the field and 6 (11.3 percent) were infected. Thus, altogether 147 F_2 plants were inoculated with the covered smut and 16 (10.8 percent) were smutted, as recorded in table 1.

These results with the second generation plants indicate that they are completely susceptible to the loose smut, corresponding in their behavior to the two parental varieties, Early Gothland and Victor. On the other hand, the results with the covered smut indicate clearly the dominance of resistance and a segregation in the second generation. The percentage of infected plants obtained in the three different experiments varied greatly, and the total number of infected plants secured was rather small.

TABLE 1. *Results with Inoculated F_2 Plants of Hybrid 16, Early Gothland × Victor*

	Inoculated with *Ustilago Avenae*			Inoculated with *Ustilago levis*		
	No. Plants	No. Inf.	Percent Inf.	No. Plants	No. Inf.	Percent Inf.
Hybrid 16: 1926*	52	52	100.0	44	8	18.1
1928	15	15	100.0	50	2	4.0
1929	—	—	—	53	6	11.3
	67	67	100.0	147	16	10.8
Early Gothland (152)	73	73	100.0	208	2	.9
Victor (126)	49	49	100.0	188	187	99.4

* These F_2 plants were grown to maturity in the greenhouse, the other two sets in the field.

The number in parenthesis after the varietal name is the seed number of the varieties used in these studies.

Data Obtained with the F_3 Generation

In addition to the second generation plants inoculated either with the loose or with the covered smut, several uninoculated plants of this generation were also grown. These plants, as well as the survivors among those inoculated with the covered smut, were available for producing third generation progenies. Out of a total of 147 F_2 plants inoculated with the covered smut 131 survived. There were, of course, no survivors among the F_2 plants inoculated with the loose smut.

Two groups of third generation progenies, then, were available for study. One of these groups descended from the uninoculated second generation plants, and these are numbered between 1 and 100; the other group descended from F_2 plants which had been inoculated with the covered smut, but had survived, and these are numbered beginning with 101.

Results with Ustilago Avenae

In view of the complete susceptibility of the second generation of the hybrid to the loose smut, relatively few F_3 progenies were inoculated with it, the data being recorded in table 2. There were grown 25 third generation progenies which had descended from the uninoculated group of F_2 plants. These included 398 individuals, of which 353 (88.6 percent) were infected. Several of the progenies gave 100 percent infection; the lowest percentage obtained was 57.1 percent.

There were 17 third generation progenies which had descended from F_2 plants inoculated with the covered smut. These contained 278 plants, of which 258 (92.8 percent) were infected. In this group also several of the progenies gave 100 percent infection, and the lowest percentage obtained with any progeny was 76.4 percent.

These results indicate clearly a high susceptibility of the third generation

TABLE 2. *Results with the F_3 Progenies of Hybrid 16, Early Gothland × Victor, Inoculated with Ustilago avenae*

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
F_3 progenies descended from uninoculated F_2 plants							
16–F_3–2	19	17	89.4	16–F_3–29	13	12	92.3
4	12	12	100.0	30	15	13	86.6
6	18	15	83.3	31	16	14	87.5
8	16	11	68.7	32	17	16	94.1
10	16	16	100.0	33	16	16	100.0
11	15	14	93.3	37	16	15	93.7
12	18	18	100.0	39	13	11	84.6
15	18	15	83.3	40	12	12	100.0
17	17	16	94.1	46	17	16	94.1
19	19	18	94.7	48	17	15	88.2
20	17	15	88.2	49	14	8	57.1
24	16	10	62.5	53	17	16	94.1
27	14	12	85.7				
25 progenies, 398 plants, 353 infected, 88.6 percent							
F_3 progenies descended from F_2 plants inoculated with *Ustilago levis*							
101	17	13	76.4	121	15	14	93.3
102	15	12	80.0	123	16	16	100.0
103	18	18	100.0	124	17	16	94.1
104	17	14	82.3	125	17	16	94.1
108	14	13	92.8	127	11	10	90.9
113	10	9	90.0	128	20	19	95.0
114	16	15	93.7	129	19	18	94.7
117	19	18	94.7	130	20	20	100.0
119	17	17	100.0				
17 progenies, 278 plants, 258 infected, 92.8 per cent							

progenies to the loose smut. They are in close harmony with those obtained in the second generation and also with the two parents.

Results with Ustilago levis

In view of the fact that the F_2 plants inoculated with the covered smut appeared to show a definite segregation of the inheritance of the resistant quality, a large number of F_3 progenies inoculated with this smut have been grown. Different series have been carried out in each of the years 1927–1931, and the data are recorded in tables 3 and 4. The results are separated on the basis of the treatment of the second generation plant. Further, the progenies are classified as resistant, those which gave no infected individuals; segregating, those in which the percentage of infection was less than 50 percent; and susceptible, those in which more than 50 percent of the individuals were smutted.

TABLE 3. *Results with the F_3 Progenies of Hybrid 16, Early Gothland × Victor, Descended from Uninoculated F_2 Plants. All Progenies inoculated with Ustilago levis*

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
			Resistant F_3 families				
16–F_3– 1*	16	0	0	16–F_3–29*	40	0	0
2*	39	0	0	30*	39	0	0
4	36	0	0	33	36	0	0
11	39	0	0	37	37	0	0
14	39	0	0	39	39	0	0
15	38	0	0	44	20	0	0
17	38	0	0	51	19	0	0
22	38	0	0	55	17	0	0
24	37	0	0	56	20	0	0
			Segregating F_3 families				
3*	38	6	15.7	32	36	2	5.5
5	35	2	5.7	35	20	2	10.0
7	34	16	47.0	36	19	1	5.2
8	38	5	13.1	40	35	3	8.5
9*	38	14	36.8	41	19	6	31.5
13	36	9	25.0	45	20	7	35.0
16*	20	1	5.0	46	39	6	15.3
18	36	3	8.3	47	19	3	15.7
20	36	10	27.7	48	32	7	21.8
21	39	1	2.5	49*	37	1	2.7
23*	38	2	5.2	52	18	2	11.1
26	40	3	7.5	53	34	3	8.8
27	40	9	22.5	54	18	2	11.1
31	38	10	26.3				
			Susceptible F_3 families				
6	35	22	62.8	42	19	11	57.8
10	31	29	93.5	43*	19	11	57.8
12	38	38	100.0	50*	18	18	100.0
19	36	35	97.2				

* The parental F_2 plants marked with the asterisk had light colored glumes, the others dark colored.

Results Obtained with F_3 Progenies Descended from Uninoculated F_2 Plants. The data obtained with these progenies are recorded in table 3. Altogether, 52 progenies were grown, most of them in the greenhouse in 1927. Further tests with additional individuals of some of them were made in 1928 and 1929.

As shown in the table, 18 of these 52 progenies gave no infection, a total of 694 plants being inoculated. There were 27 progenies which gave less than 50 percent infection, the percentages ranging from 2.5 to 47 percent. In a few of these progenies only one infected plant occurred. The 7 re-

TABLE 4. *Results with the F_3 Progenies of Hybrid 16, Early Gothland × Victor, Descended from F_2 Plants which Had Been Inoculated with Ustilago levis. All Progenies Inoculated with Ustilago levis*

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
Resistant F_3 families							
16–F_2–102, 111*, 112, 119, 120, 121, 122*, 124, 129, 130, 131, 134, 135, 139, 140, 142, 143*, 145, 146*, 149, 154*, 155, 156, 157, 160, 163, 165, 166, 169*, 170, 172*, 179, 184, 187, 189, 193*, 201, 202, 203, 206*, 209*, 211*, 214							
43 progenies, 1396 plants, none infected							
Segregating F_3 families							
101	18	5	27.7	168*	49	5	10.2
103	36	12	33.3	171	45	10	22.2
104*	34	3	8.8	173	48	1	2.0
106	20	3	15.0	174	25	4	16.0
107*	18	5	27.7	176	20	3	15.0
108	40	13	32.5	177	23	8	34.7
109	8	2	25.0	178	21	3	14.2
113*	18	4	22.2	180	24	2	8.3
114*	20	1	5.0	181	24	3	12.5
115	14	2	14.2	182	24	3	12.5
116*	6	1	16.6	183	23	1	4.3
117*	31	13	41.9	185	22	5	22.7
123*	15	2	13.3	186*	24	3	12.5
125	32	4	12.5	188	22	6	27.2
127	19	1	5.2	190	23	4	17.3
128*	18	5	27.7	191	23	4	17.3
132	36	5	13.8	192	25	2	8.0
136	17	1	5.8	194	24	5	20.8
137	44	2	4.5	195	21	3	14.2
138	46	15	32.6	196	22	2	9.0
144	47	12	25.5	197	23	10	43.4
147	47	5	10.6	198	23	6	26.0
150	44	10	22.7	199	24	6	25.0
151	47	17	36.1	200	23	10	43.4
152	25	6	24.0	204*	23	2	8.6
153	46	10	21.7	205	23	1	4.3
158	40	6	15.0	207	23	3	13.0
159	48	4	8.3	208	24	4	16.6
161	45	7	15.5	210	25	5	20.0
162*	43	11	25.5	212	23	9	39.1
164	48	13	27.0	213	25	6	24.0
167	49	7	14.2	215*	24	5	20.8
64 progenies, 1834 plants, 351 plants infected, 19.1 percent							

* The parental F_2 plants marked with the asterisk had light colored glumes, the others dark colored.

maining progenies were classified as belonging in the susceptible group, the percentage of infection being greater than 50; 2 of these progenies gave 100 percent smutted plants, the other 5 ranging from 57.8 to 97.2 percent.

These results indicate clearly that three types of families are obtained—resistant, segregating, and susceptible.

Results Obtained with F_3 Progenies Descended from F_2 Plants Inoculated with Covered Smut. Altogether, 107 progenies of this group have been grown during the years 1927–1931. The data are recorded in table 4. Of these 43 gave entirely negative results, a total of 1396 plants being inoculated. The remaining 64 progenies gave percentages of infection varying from 2 to 43.4 percent; these progenies contained 1834 plants, of which 351 (19.1 percent) were infected. The experiments were repeated with some of the families in the successive years but, for the most part, they behaved in a similar fashion. In a few cases, however, a progeny which gave negative results one year showed a few susceptible plants in the following test. The results indicate clearly that two types of progenies are present—resistant and segregating. No families which could be classified as susceptible were obtained, since in no case was the percentage of infection above 50 percent.

It is interesting to consider these progenies in relation to the particular F_2 plants from which they were grown. As already noted, 44 F_2 plants inoculated with the covered smut were grown in 1926, and 8 of these (18.1 percent) were infected. From these surviving plants, 31 third generation progenies were grown, 13 of which proved to be resistant and 18 segregating. In 1928, 50 second generation plants were grown, of which 2 (4 percent) were smutted. Third generation progenies were grown from 46 of these surviving second generation plants and 20 of them proved to be completely resistant and 26 showed segregation. Thus in both the second and third generation of this set of plants we find an unusual preponderance of resistance. Finally, 53 second generation plants were grown in 1929 and 6 (11.3 percent) were infected; from these survivors, 30 third generation progenies were grown, 10 of which were resistant and 20 segregating.

Data Obtained with the F_4 Generation

The investigations were continued with 80 fourth generation progenies selected from 10 resistant F_3 families and inoculated with the covered smut (table 5). From each of the F_3 families seed was taken from 5 to 9 different plants in order to grow the fourth generation. Of these fourth generation progenies, 15 were descended from 2 F_2 plants which had also been inoculated with the covered smut. The remaining 65 progenies came from 8 uninoculated second generation plants, although the third generation had been inoculated and was completely resistant. Thus, altogether, 10 different second generation plants were represented by descendants in the fourth generation.

There were included in these 80 F_4 progenies a total of 1538 individual plants inoculated with the covered smut. Not one of these plants was infected. Thus the resistance shown in the third generation is also evident in these fourth generation progenies.

TABLE 5. *Results with Some F_4 Progenies of Hybrid 16, Early Gothland × Victor, Descended from F_3 Progenies Resistant to Ustilago levis. All Progenies Inoculated with Ustilago levis*

Number of F_3 Progeny	Number of F_4 Progenies Grown	Number of F_4 Plants Grown	Number Infected
1	9	175	0
2	9	161	0
11	7	135	0
24	9	177	0
29	9	177	0
30	8	152	0
33	5	96	0
37	9	177	0
111	7	134	0
122	8	154	0
	80	1538	0

F_3 progenies 1, 2, 11, 24, 29, 30, 33, 37 grown from uninoculated F_2 plants.
F_3 progenies 111, 122 grown from F_2 plants inoculated with *Ustilago levis*.

INHERITANCE OF GLUME COLOR AND ITS RELATION TO THE INHERITANCE OF RESISTANCE TO COVERED SMUT

Perhaps the most obvious difference between the two parental varieties, Early Gothland and Victor, is the color of the glumes of the grains. These are white or pale yellow in Early Gothland, and brownish black to black in Victor. The data on the glume color of the second generation plants are recorded in table 6.

TABLE 6. *Glume Color of the F_2 Plants of Hybrid 16, Early Gothland × Victor*

F_2 Plant No.	Year Grown	Total No. of Plants	Dark Hulls	Light Hulls	Percent with Light Hulls
F_2–1–56 Not Inoculated.....	1926	56	43	13	23.2
F_2–101–136 Inoculated.....	1926	34	22	12	35.2
F_2–137–185 "	1928	49	42	7	14.2
F_2–186–226 "	1929	40	32	8	20.0
		179	139	40	22.3

The F_2 plants No. 1–56 and 101–136 were grown to maturity in the greenhouse and the others in the field. The glume color of the inoculated F_2 plants which proved to be susceptible, of course, was not obtained, since these plants failed to produce any seed. The uninoculated F_2 plants consisted of 13 out of 56 with light hulls (23.2 percent). The F_2 plants which had been inoculated, but survived, had 27 with light hulls out of a total of 123 (21.9 percent). Thus there were no important differences in the percentage of plants with light hulls in the two groups.

As noted in the table, the inoculated F_2 plants were grown at three different times. The plants numbered 101–136 contained a relatively high

proportion of light hulled plants, while the other two groups, especially those numbered 137–185, contained an excess of the dark hulled plants; the average, however, was practically the same as for the group of uninoculated individuals.

The color of the hulls of the F_2 plants in relation to the behavior of the F_3 progenies was found to be as follows:

	Resistant		Segregating		Susceptible	
	Dark Hulls	Light Hulls	Dark Hulls	Light Hulls	Dark Hulls	Light Hulls
F_2–1–56.....	14	4	22	5	5	2
F_2–101–226....	32	11	41	13	0	0

Third generation progenies were not grown from all of the F_2 plants. Thus we find, descended from both dark and light hulled plants, the different types of progenies—resistant, segregating, or susceptible, and there are no marked departures from the expectations in the occurrence of the different groups. There are, therefore, no clear indications of any correlation whatever between the inheritance of the color of the hull and resistance or susceptibility.

Discussion and Summary

The results obtained with the hybrid between Early Gothland and Victor are interesting in the light of those secured with other types of oat crosses. When Black Mesdag is crossed with a susceptible variety such as Hull-less, resistance to both loose and covered smut is dominant, and segregation occurs in a ratio more or less approximating three resistant to one susceptible (3). Further, by far the greatest number of the third generation progenies showed a similar behavior to both loose and covered smut. Thus there was a marked parallelism in the inheritance of resistance to both smuts in the various progenies.

I have recently published data on the inheritance of smut resistance in crosses between Early Gothland and Monarch (6). These two varieties differ in that Early Gothland is very susceptible to loose smut and resistant to covered, while Monarch is exactly the reverse. Data were obtained on the second, third, and fourth generations, different sets of plants being inoculated with loose and covered smut. The most obvious fact in the results was the absence of similarity in the behavior of the various F_3 progenies to the two smuts. The data indicated clearly that the factors for resistance to loose and covered smut are independently inherited in these hybrids. A third generation progeny resistant, segregating, or susceptible to the loose smut might belong to any one of these classes in its behavior to the covered smut.

In the hybrid between Early Gothland and Victor, one parent is susceptible to both smuts, while the other is susceptible to loose smut and resistant to the covered. The results with both the second and third generation plants show practically complete susceptibility to the loose smut. All of the 67 F_2 plants inoculated became infected, and a very high percentage of infection was also obtained with the third generation progenies grown. One group of 25 progenies descended from uninoculated F_2 plants; these contained 398 individuals, of which 353 (88.6 percent) were infected. Another group of 17 third generation progenies which had descended from F_2 plants inoculated with the covered smut was grown; these contained 278 plants, of which 258 (92.8 percent) were infected.

The question may be raised as to whether there is any significance in the failure of all of these third generation plants to become infected. In a considerable number of the families, all of the individuals were smutted, while in the others, one or more plants remained normal. It may be that these merely escaped infection, although the methods of inoculation were highly effective, as shown by the results secured with the parental varieties. It will be necessary to grow the descendants of these plants in order to determine whether they are merely chance escapes or whether they possess some real inherited resistance to the loose smut.

The results obtained with the covered smut show clearly that resistance is dominant and that segregation occurs in the second generation. The facts obtained for the third generation are more or less in harmony with those secured for the second. Further, resistant third generation progenies have also given rise to entirely resistant fourth generation families.

There is, however, a marked preponderance of F_2 plants resistant to the covered smut. Altogether, three different sets were inoculated and grown at different times. The highest percentage of infection obtained was 18.1 percent, 8 plants out of 44 inoculated being infected. In the other two experiments, the number of smutted plants was much lower, and the combined results indicate only 16 infected individuals (10.8 percent) out of a total of 147.

Further, this preponderance of resistance also appeared in the third generation. If the third generation progenies are grown from uninoculated F_2 plants, we find a high proportion of resistant ones. There were grown 52 progenies of this group, of which 18 were entirely resistant, 27 segregating, and only 7 susceptible. In the other group of F_3 progenies descended from inoculated F_2 plants, we again find a high proportion of resistant families as compared with the segregating. A total of 107 of these progenies was grown, 43 being resistant and 64 segregating. No susceptible progenies were found in this group, a result which would be expected unless a susceptible F_2 parental plant escaped infection.

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THE MORPHOLOGY AND DEVELOPMENT OF *ENTOMOPHTHORA FUMOSA*

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INTRODUCTION

Our knowledge of the morphology of the Entomophthoreae dates from the description by Cohn in 1855 of the vegetative structure within the host and the asexual reproduction of *Empusa Muscae.* Brefeld (1870–77) carried the investigations upon *Empusa Muscae* a little farther, describing the germination of the conidia and the growth of the fungus within the host by means of short, thick cells. He also made an extensive series of observations on *E. sphaerosperma* tracing the development of the organism from infection, through the filamentous vegetative growth, to the production of conidia and to the formation of thick walled resting spores, the development of which follows the septation and anastomosing of the mass of hyphae filling the host.

Nowakowski (1877) described the vegetative growth and conidial development of *E. curvispora* and *E. ovispora.* He recorded the formation of the zygospores to be brought about by means of fertilization tubes similar to those found in *Spirogyra.*

Thaxter (1888), in his monograph on the Entomophthoreae of the United States, gave the results of morphological studies based on a large number of new forms as well as old ones. He recorded the variations which he found to occur in the vegetative structure, in conidiophore development, and in the methods of the formation of resting spores, when present. Cavara (1899) was the first, however, to point out the differences between the multinucleate conidia of *Empusa Muscae* and the uninucleate conidia of *E. Delpiniana.*

Olive (1906) found the vegetative structure of *E. Culicis* to consist of two types of hyphal bodies, each of which produces its characteristic conidiophore bearing a terminal 2-nucleate conidium. He found a different condition to exist in *E. Sciarae* both in the vegetative structure and in conidial development.

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Riddle (1906) found that, preceding the death of the host, segmentation of the vegetative hyphae of *E. Grylli* occurred and single conidiophores were developed from each hyphal segment. He recorded two methods of zygospore formation in *E. americana:* either two hyphal bodies fuse and the young zygospore buds out at the point of fusion, or a lateral fusion of two hyphal bodies takes place and the zygospore buds out from one of the fusing cells.

Speare (1921), using the material *in toto*, described the vegetative development of *Entomophthora fumosa* within the host, by means of 4-nucleate hyphal bodies. He gave an account of conidiophore formation from the hyphal bodies, and the appearance of the black, spherical, resting spores. He observed that no external growth appeared when resting spores were formed within the individual hosts. This condition, however, is not true of all members of the Entomophthoreae.

Methods

The material for the study of *Entomophthora fumosa* on *Pseudococcus citri* Risso, the citrus mealy-bug, was collected at the end of June, 1926, by Mr. W. A. Kuntz at the Citrus Experimental Station, Lake Alfred, and at Eagle Lake, Florida. All the infected mealy-bugs were fixed in formo-acetic-alcohol killing fluid by Mr. Kuntz. After remaining in this killing fluid about twenty-four hours, the material was dehydrated by running through grades of alcohol, cleared in xylol or chloroform, and embedded in paraffin.

The sections were cut 5 to 7 microns thick and fastened to slides with albumen. These were stained with Flemming's triple stain (safranin, gentian-violet, and orange G) or with Heidenhain's iron haemotoxylin which was found to be the most satisfactory stain for the desired results. After staining, the slides were dehydrated, cleared with clove oil, and mounted in Canada balsam.

Observations and Discussion

Vegetative Stage

My observations are concerned with the division of the hyphal bodies and the consequent vegetative development of the fungus within the host, the formation of the conidia, and the development of the zygospores of the single form, *Entomophthora fumosa*, a member of the family Entomophthoreae, and parasitic on *Pseudococcus citri* Risso, the citrus mealy-bug. Speare (1922) regarded this species as being distinct from anything that had previously been described, because of the size and shape of the conidia and the resting spores, which he considered unlike those of any other known form. I shall continue to use the name *Entomophthora fumosa* which was suggested by Speare. If, however, a species were to be determined entirely on spore size, it would be questionable as to whether the

form I have worked with is not a different species from that described by Speare. I find the size of the conidia to be 6.7 to 7.3 microns by 13.4 to 14.7 microns, and the zygospores 10 to 12 microns, in contrast with the size given by Speare which is 8 to 10 microns by 16 to 20 microns for the conidia and 15 microns for the zygospores. I also find that the entire contents of the hyphal body and conidiophore do not pass into the single conidium as Speare indicated. This condition will be described in detail later on in the paper.

In some hosts which I have examined, the infection is found to be in a very early stage. Contrary to conditions in the vegetative form described by Brefeld for *E. sphaerosperma*, by Sorokin for *E. conglomerata*, by Riddle for *Empusa Grylli*, and by Olive for *Empusa Aphidis* and *E. Sciarae*, no mycelium is found in the vegetative stage of *Entomophthora fumosa*. In the very early stages of infection (Pl. XVI, fig. 41) only a few short, thick, usually spherical fragments of the fungus, to which Thaxter (1888) gave the name "hyphal bodies," are found scattered singly or in small groups near the periphery of the insect just beneath its chitin wall. In a slightly later stage, a few hyphal bodies are found in the body cavity scattered among the host tissues, as well as near the periphery. Possibly these had been carried in the blood stream, as Speare suggests, since they are often found singly, or if there are several present, they are distinctly separated and not in the form of a mycelium, or segments of a mycelium, which have become separated and rounded up to form hyphal bodies. Goldstein (1929) found the latter to be true for *Massospora cicadina*, which has branching hyphae present in the early vegetative stages. These hyphae later became septate and the two-nucleate mycelial fragments rounded up to form hyphal bodies, after the host tissues had become disorganized.

As the fungus multiplies, hyphal bodies are found scattered throughout the body cavity surrounding the soft internal tissues of the diseased mealy-bug. Speare reported that as far as he was able to determine, the fungus lived primarily in the blood, absorbing nourishment from it and the other body fluids. After a time the hyphal bodies have multiplied rapidly and a breaking down of the muscles and other softer tissues takes place, leaving only the heavily chitinized structures surrounding a more or less compact mass of hyphal bodies.

The hyphal bodies are more or less pressed together as they increase in number (fig. 42), but at no time is a true vegetative mycelium present. In accordance with Thaxter's observations of many of the Entomophthoreae, the hyphal bodies present in the host at the time of its death differ somewhat in both size and shape, in that they are slightly larger and more regular, being spherical in most cases, than those present in the earlier stages of the fungus. This is probably due to the rapidity with which the hyphal bodies increase in number in the vegetative stage of the disease. Those cells which are rapidly undergoing division would naturally have a softer and

more delicate wall than those hyphal bodies which are present at the end of the vegetative period when no more cell division is to take place. Those extremely thin-walled cells found early in the life of the fungus, therefore, assume irregular shapes if pushed together.

The hyphal bodies are from 8 to 10 microns in diameter, the largest being those which are ready to produce conidiophores. For the most part they are spherical (fig. 41), except when closely packed together or pressed against some of the insect tissue. In a few insects examined, the hyphal bodies are so closely packed together within the host wall that they appear angular and not spherical. The hyphal bodies which are to produce conidiophores (fig. 43), are more regularly spherical than when the infection is in its early stages. The cell wall is extremely thin and in many instances, when the hyphal bodies are rapidly increasing in number, it appears to be hardly more than a membrane. The slightly thicker and more definite wall forms at the end of the vegetative period when further division of the hyphal bodies is at an end. Speare noted that the wall was so thin and plastic that, when he pressed one of the hyphal bodies with the point of a needle, the cell became irregular or often dumb-bell shaped.

The hyphal bodies are filled with finely granular cytoplasm which is very vacuolate, some of the vacuoles being rather large and others very small. As is to be expected, the cytoplasm is somewhat denser at the periphery of the fungus cell and around the nuclei than it is elsewhere.

The resting nucleus is small and usually contains a single large nucleole (which in my preparations stained a deep red when Flemming's triple stain was used), although a few nuclei were observed to have two or three nucleoles. Around the nucleole and occupying a relatively large part of the nucleus, blue-staining chromatin is found in the form of minute, closely packed granules, the whole being bounded by a definite nuclear membrane. The nuclei are spherical in both the hyphal bodies and conidia, but often appear to be slightly ovoid in the conidiophores. The number of nuclei present in each hyphal body is typically four. These are usually regularly spaced near the center of the hyphal body. Occasionally a very large hyphal body is found which contains more than the usual number of nuclei. The nuclei present, however, are in powers of four and in one very large hyphal body thirty-two nuclei were found. No explanation can be given which accounts for this increase in size of the cells and in the number of nuclei present, except that, following repeated nuclear division, the cells increased proportionately in size without cell division.

The hyphal bodies reproduce rapidly so as to fill the entire body cavity and legs of the insect. Thaxter described the development of *Empusa* within the host as mainly a process of repeated budding and growth of hyphal bodies. Speare reported that the hyphal bodies of *E. fumosa* multiplied by a budding process. Olive (1906) was of the opinion that cell division in *Empusa*, as in many of the lower plants, took place entirely

independently of nuclear division and apparently remote from nuclear control, since no cell plate was ever formed at the close of nuclear division. I am of the opinion that in *E. fumosa* the division of the hyphal bodies follows immediately after the division of the nuclei.

I find that the division of the four nuclei in the hyphal body is simultaneous (Pl. XIV, figs. 1, 2). As is to be expected, not all of the hyphal bodies present in the host undergo nuclear division at the same time, but only occasional cells are found in which this phenomenon is taking place. The material was fixed in formo-acetic-alcohol which is not always a favorable cytological killing fluid. It was impossible to stain the material with Flemming's triple stain to make a minute detailed study of the nuclei, except in so far as was necessary to bring out related features. The nuclear division in the hyphal bodies appears to be a normal mitosis. Riddle found the spindle of *E. americana* to be intranuclear. If the spindle of *E. fumosa* is intranuclear, the nucleus elongates greatly during the early stages of division, for the spindle is much larger than the resting nucleus and the four dividing nuclei, which are very conspicuous, occupy a large part of the hyphal body.

In the earlier stages of nuclear division there appeared to be a large number of minute chromatin-like granules, scattered more or less regularly in the center of the cell and occupying somewhat the same positions as had the resting nuclei. Later stages, which might be considered as extending from the late anaphases into the telophase, occur more frequently. In the later stages (figs. 1, 2) large conical masses of chromatic material, probably due to an aggregation of minute granules at the poles, are found at both ends of the spindle and occasionally two to four larger definite granules of chromatic material are found attached to the spindle in such a way as to resemble an equatorial plate. Later stages are found where these larger granules have divided and two are being drawn toward each conical mass of chromatic material at the ends of the spindle of the dividing nucleus.

I observed no centrosomes in the nuclear division, such as figured for *Empusa Sciarae*. The uppermost portion of the conical masses at the ends of the spindle resembles in some respects the "pole-plates" figured by Olive (1907) in the nuclear division in *Basidiobolus*. These are present even in the earlier stages when the granules are numerous and scattered, before the development of the definite conical masses. As to what rôle the nucleole plays in the division of the nucleus, I am not able to state. When the granules have come very close to the polar masses the spindle seems to split into two half spindles which begin to move apart (Pl. XIV, fig. 3). The cone-shaped mass of chromatin and the two definite granules, which accompany each, later become surrounded by a finely granular nucleoplasm. The daughter nuclei, which have not yet reached the final resting stage, begin to distribute themselves more or less regularly near the ends of the hyphal body (figs. 4–6), which has become somewhat ellipsoid

and slightly larger during the process of nuclear division. Later four daughter nuclei, which have not completely returned to the resting stage in some instances, are found more or less regularly spaced at each end of the elongated hyphal body.

At the time of the separation of the daughter nuclei, slight indentations from the periphery, near the center, of the slightly elongated hyphal body are observed (figs. 3, 4). The process starts at the periphery of the fungus cell due to the constriction of the plasma membrane. This phenomenon was recorded by Lutman (1911) as the beginning of cell division for *Closterium*. In this case, however, a cell wall was deposited inside the furrow as it progressed.

The constriction is progressive from the surface of the hyphal body inward, the furrow deepening in a radial direction (figs. 4–10). As the constriction progresses the contraction of the protoplasm becomes rather conspicuous (figs. 8–10). The furrow opens somewhat and the two masses of protoplasm appear to round up (figs. 9–10). The furrow continues to grow radially inward, ultimately separating two daughter hyphal bodies, which are like the parent cell in every way except as to size.

Preceding and accompanying this ingrowth of the plasma membrane, a thin layer of rather dense cytoplasm appears along the innermost parts of the furrows. Contrary to Harper (1899, p. 506), the ingrowth is not simply a deep, narrow furrow, which cuts across the cell with the cell wall of fungus cellulose forming later inside the furrows. In my preparations, the extremely thin, delicate walls are not easy to differentiate, and in some instances seem to be hardly more than a membrane. The wall of the hyphal body is so thin and plastic that by adhesion to the plasma membrane, it is pulled in with the membrane by whatever force it is that causes the cleavage of the cell. I am of the opinion, however, that, unlike the condition described by Dr. Harper and that found in certain other fungi, in *E. fumosa* a delicate wall is formed simultaneously with the cleavage furrow and not afterward.

The cleavage-furrow starts in a definite region of the plasma membrane nearly equidistant from the ends of the slightly ellipsoidal hyphal body. Apparently the plasma membrane is stimulated to action by some phase of the nuclear division, since the beginning of the furrow is seen before nuclear division is complete. Probably by the time the constriction of the hyphal body is complete, the nuclei have returned to a resting condition, although in some instances the nuclei have a granular appearance (fig. 10). Some of the nuclei appear to be still in the late telophase; this may be due, however, to fixation. When the daughter hyphal bodies have separated they are about one-half the size of a mature fungus cell. This method of the division of the hyphal bodies is repeated until all the insect tissues are broken down and absorbed by the fungus as a source of nourishment, and only the chitin walls of the host remain surrounding the mass of hyphal bodies.

Reproduction

The formation of new hyphal bodies ceases with the death of the host. Either just preceding or after the death of the insect, the hyphal bodies give rise either to conidiophores, which bear conidia, or to zygospores. The type of spore produced depends upon factors which are not clearly understood. Contrary to the condition found in *Empusa Muscae*, the resting spores and conidia are never found either simultaneously or consecutively on the same individual host. Speare (1921) found this same condition to exist in *Massospora cicadina* Peck. Both spore forms arise from hyphal bodies which behave differently in each instance.

At the beginning of the fructifying condition, all the hyphal bodies present in the host appear slightly larger than those found during the active vegetative growth and are, for the most part, isodiametric, unless they are so abundant and are so closely packed together that they become angular as a result of the pressure. The latter condition was found in only a few insects, however. When the hyphal bodies germinate to produce conidiophores, a small dark-staining area is seen at the point on the periphery from which the hypha will develop (fig. 11). At this time the wall of each hyphal body appears to be slightly thicker than that found in those cells of the vegetative stage which are continually undergoing division. The cytoplasm is of a more or less definite alveolar structure and finely granular.

The hyphal bodies near the wall of the insect host appear to germinate more or less simultaneously. There follows a progressive germination of the hyphal bodies lying farther inside the host wall. As long as suitable conditions of temperature and moisture are present, the hyphal bodies remain active and give rise to conidiophores, which are able to force their way between the collapsed conidiophores formed by hyphal bodies lying near the periphery, to the exterior to form and discharge conidia (Pl. XVI, figs. 44, 45).

A small protuberance with a densely staining tip is pushed out from the periphery of each hyphal body (Pl. XIV, figs. 11–16) and is oriented toward the wall of the insect. The hypha continues to elongate and pushes through the body wall of the insect. The diameter of the hypha varies slightly, depending upon the available space it has in which to grow between the adjacent hyphal bodies, and upon the length of the tube formed. During this growth, the cytoplasm is vacuolate at the basal portion of the hypha and there seems to be a movement of the cytoplasm up into the rounded terminal portion of the hypha.

One nucleus at a time moves into the developing hypha until all four nuclei have migrated from the hyphal body (figs. 16–20). In the meantime, the hyphal body has become decidedly vacuolate and, in some instances where the hyphae have grown quite long and are four-nucleate, the cytoplasm, probably due to the contraction of the plasma membrane, appears to be forced into the young conidiophore (fig. 20). This would indicate

that all of the cell contents may pass into the hypha, leaving it attached at the base to an empty vesicle. This condition was found to occur in several instances, but the empty hyphal bodies are soon crushed as the hyphae from farther inside grow to the outside of the insect. The nuclei in the hypha, as was the case in the hyphal body, show a strikingly regular spacing up to the beginning of the formation of the conidiospore (Pl. XV, figs. 22, 23; Pl. XVI, fig. 44), when there is a condensation of heavy granular cytoplasm and a closer grouping of nuclei at the somewhat enlarged club-shaped tip of the conidiophore. More than four nuclei have been found in the conidiospores (Pl. XV, figs. 23, 26, 33), and nuclear division in the hyphae has been observed in a few instances (fig. 1).

As the conidiophores emerge from the insect wall, they become swollen and appear in cross-section somewhat larger in diameter than at the basal portion in the insect's body. The conidiophores are simple and nonseptate until the spore is cut off. They push through the body wall of the aphid at any point, and are not limited to the weaker places in this wall, as is true of certain other Entomophthoreae.

At the terminal portion of each conidiophore, the cytoplasm becomes denser and a small protuberance which has a diameter of about half that of the conidiophore is pushed out (figs. 24, 25). At the apex of this narrow sterigma a swelling is formed which, by continued enlargement, receives part of the cytoplasm of the conidiophore and typically four nuclei, which migrate into it, one at a time, from the enlarged terminal portion of the conidiophore (figs. 26–33). The swollen portion continues to increase in size, the cytoplasm becomes granular, and the four nuclei are grouped more or less regularly in the young conidium.

The mature conidium is cut off from the conidiophore by the formation of a wall and is forcibly thrown off from the hypha, due to the swelling and bursting of a large vacuole in the terminal portion of the conidiophore. No gelatinous collar was observed attached to the mature spores. Speare reported that in the field, the conidia may be thrown to a distance of five millimeters.

The mature conidia (fig. 34) are from 6.7 to 7.3 microns by 13.4 to 14.7 microns in size. Speare recorded the size of the conidia for *E. fumosa* as 8 to 10 microns by 16 to 20 microns. These spores are considerably larger than the largest mature conidia found in the material studied. The conidia are typically more or less fusiform, although occasionally elliptical, tapering rather abruptly toward both the sharply rounded apex and the base or papilla which is clearly visible. The mature spores show an even, finely granular cytoplasm and four nuclei, more or less regularly spaced, surrounded by a much thickened wall.

Contrary to Riddle's results in *E. geometralis* and Speare's observations of *E. fumosa*, I find that in *E. fumosa* nuclear division does occur in the conidiophore during its development. Nuclear division was observed in

several developing hyphae, and in nearly all mature conidiophores as many as seven or eight nuclei were to be found. More may have been present but, due to the twisting and turning of the hyphae to reach the outside of the host, it was not often possible to trace a long hypha from its base to its tip. After four nuclei have entered the spore and the mature conidium has been cut off from the conidiophore, several nuclei and an abundance of cytoplasm are still left in the swollen tip of the hypha (fig. 33). Back of this swollen portion containing the remaining protoplasm a wall appears to have been formed, separating it from the more or less collapsed, shrunken, disintegrating hyphal tube. Since the conidia just cut off have four nuclei and that portion of the protoplasm left in the swollen tip of the conidiophore contains usually the same number of nuclei, it is possible that two sets of conidiospores may be formed in *E. fumosa* from the same hyphal strand instead of having the remaining protoplasm merely break down and disintegrate.

The mature conidia are probably capable of immediate germination if there is sufficient moisture present. Speare reported finding small elliptical secondary conidia arising from one to five slender, capillary-like conidiophores from each primary conidium. I have found no secondary conidia in my material. This, however, may be a result of both the time of collecting and the method of fixation. Germinating conidia, each of which produces from the side of the spore a single germ tube, are frequently found (fig. 35). The germ tubes are of variable length. One short germ tube, containing the four nuclei and the cytoplasm of the spore, was found which was just beginning to penetrate the chitinized wall of the host (fig. 36). In *E. fumosa*, as in other species, *Empusa Muscae* and *E. macrospora*, numerous detached spherical cells, hyphal bodies, are formed due to the penetration of the walls of the insect by the germ tube. The primary four-nucleate hyphal bodies of *Entomophthora fumosa* formed from the protoplasm of the germinating conidia divide and multiply rapidly, as has been described earlier in this paper, and repeat one part of the life history of the fungus.

The insect hosts which contain thick-walled resting spores in various stages of formation occur infrequently, for of the many insects I have examined I found only a few individuals in which these spores are present. No doubt the formation of the resting spores is influenced to some extent by the season of the year in which the material is collected. The material I have was collected near the end of June. This may be one factor responsible for the limited number of insects containing resting spores, which are usually considered to be the means by which a fungus lives through a period of time not favorable for active growth. In every instance the insect hosts containing resting spores are smaller in size than those which contain either hyphal bodies or conidiophores. Host tissue was found still to be present in varying amounts, even though some of the resting spores

appeared to be mature. This is contrary to the condition usually found when conidiophores are being produced.

Only one method of zygospore formation in *E. fumosa* was observed. This is similar to the method of the formation of resting spores in *E. Fresenii* as it has been described by Thaxter. The four nuclei of two spherical hyphal bodies, lying side by side in the insect host, undergo one division so that each cell contains eight nuclei. All eight are to be seen only by careful focusing with the microscope. Accompanying this nuclear division there is no corresponding increase in the size of the hyphal bodies such as was found when the hyphal bodies were going to divide. The walls of the conjugating hyphal bodies break down at the point of contact. One nucleus from each hyphal body comes to lie very close to the point of fusion (fig. 37) where a slight swelling is soon noted. The swelling increases in size and into this bud (the young zygospore) pass a portion of the cytoplasm and a nucleus from each hyphal body (fig. 38). The nuclei are slightly elongated due to the smallness of the passage way into the developing zygote. With the continued enlargement of the young zygospore, which is spherical in shape, the two nuclei persist but seem to draw apart rather than to form a fusion nucleus (fig. 39). As the zygospore matures a cross wall is formed, cutting off the spore from the two partially empty hyphal bodies, and two thickened walls are laid down around the spore, the exospore being very dark and the endospore of a hyaline nature. The mature zygospores are spherical in shape and 10 to 12 microns in diameter, in comparison with the zygospores of *E. fumosa*, which Speare found to be 15 microns in diameter.

As to what becomes of cytoplasm and nuclei which remain in the gametes after the formation of the young zygospore, I am not prepared to state. Indications in some of the material lead me to believe, however, that the nuclei remaining in the gametes disintegrate and finally disappear. When the zygospore is mature, portions of the hyphal body walls may be seen for a time attached to its base. These also disappear later and the thick-walled spore has only a slight hyaline protuberance or papilla showing the point of attachment of the hyphal bodies (fig. 40).

Summary

No mycelium is ever observed in the vegetative growth of this fungus but instead, four-nucleate hyphal bodies fill the body of the host.

The four nuclei of the hyphal body undergo division simultaneously. Nuclear division is followed by the constriction of the fungus cell to form two daughter cells. The division of the hyphal bodies is by constriction and not by budding.

The entire contents of the hyphal body pass into the conidiophore. Nuclear division takes place in the conidiophore as well as in the hyphal body, so that more than four nuclei are usually in the developing conidiophore. The conidia, always formed outside the body wall of the host, are four-nucleate.

Resting spores are produced within the host as a result of the fusion of two adjacent hyphal bodies. The resting spores are binucleate and very thick-walled.

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EXPLANATION OF PLATES

Figures 1, 4, 5, 6, 9, 10, 37–40, were made with the aid of a Zeiss camera lucida together with Zeiss 1 : 40 aperture apochromatic objective, combined with the compensating ocular 12. × 1900. Figures 2, 3, 7, 8, 11–36 were made with the aid of an Abbe camera lucida together with a Leitz 1/16 oil immersion objective and compensating ocular 5. × 2000.

PLATE XIV

FIGS. 1, 2. Advanced stages of nuclear division in hyphal body.

FIG. 3. Separation of halves of spindles and movement apart with increase in length of hyphal bodies.

FIG. 4. Distribution of daughter nuclei in ends of hyphal bodies with cleavage furrows beginning to form.

FIGS. 5–10. Continued ingrowth of cleavage furrows until hyphal body is nearly divided.

FIGS. 11–15. Hyphal bodies each having four nuclei and showing the development in length of hyphal tube with accumulation of material at tip.

FIG. 16. Hyphal body containing three nuclei and showing the passage of one nucleus into the young conidiophore.

FIG. 17. Hyphal body having three nuclei and in young conidiophore one of two daughter nuclei derived from nucleus as seen in FIG. 16 in stage of division.

FIG. 18. Passage of second nucleus from hyphal body into conidiophore.

FIG. 19. Hyphal body containing one nucleus and three nuclei in tube, which have come from attached hyphal body.

FIG. 20. Young conidiophore showing all of the nuclei of hyphal body with no nuclear division having taken place.

FIG. 21. Division of nucleus in hyphal tube or conidiophore.

PLATE XV

FIG. 22. Club-shaped tip of young conidiophore preceding emergence from insect wall and showing equal spacing of nuclei.

FIGS. 23–26. Variations in number of nuclei observed in tip of conidiophore.

FIG. 24. Formation of sterigma at tip of conidiophore.

FIG. 25. Beginning of young conidium as slight swelling at apex of sterigma.

FIG. 26. Developing young conidiospore at apex of young sterigma. Nuclei grouped in tip of conidiophore prior to migration into developing spore.

FIG. 27. Developing conidiospore showing passage of first nucleus.

FIGS. 28–29. Developing conidiospore showing migration of single nucleus into spore.

FIGS. 30–33. Movement of other nuclei into young conidiospore to give the typical number, four.

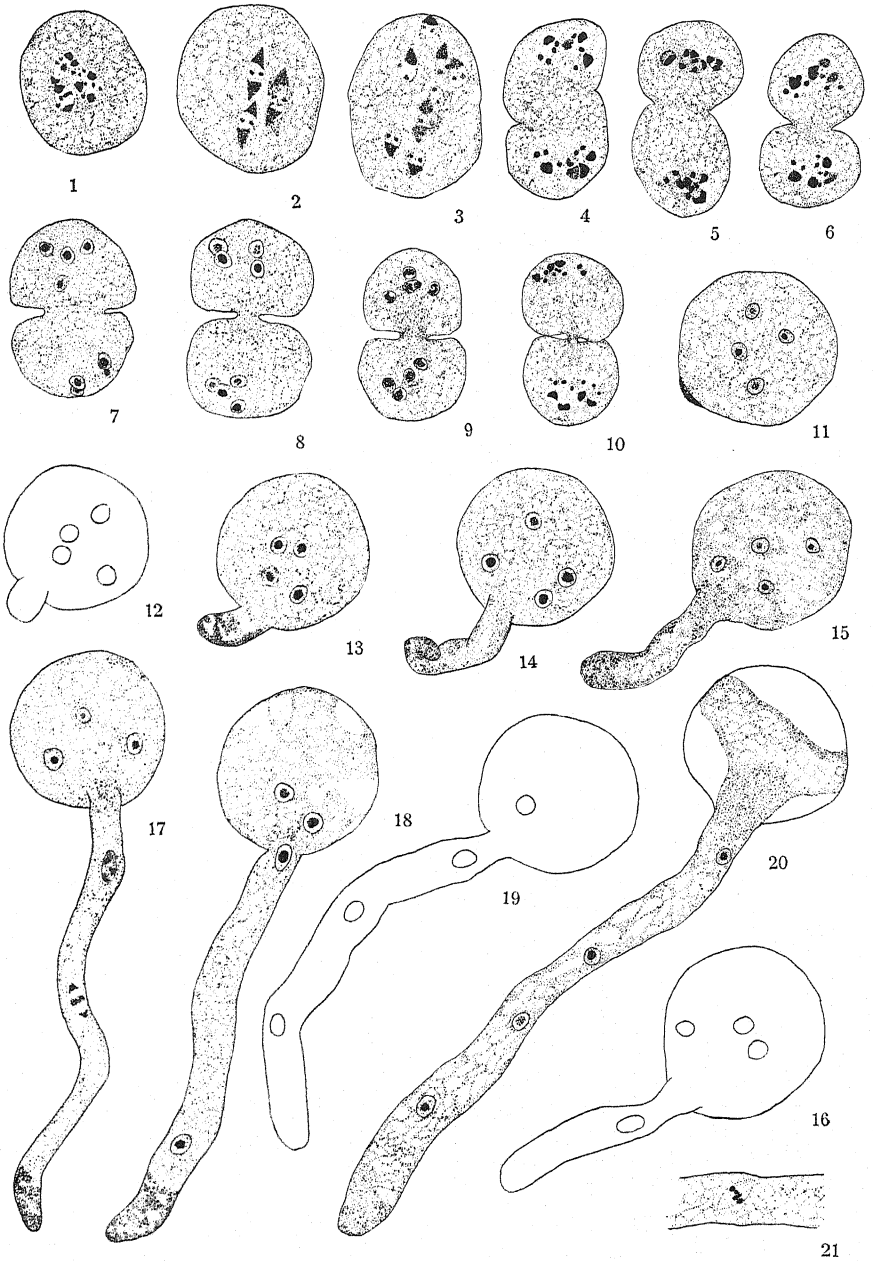
FIG. 33. Vacuole at base of young four-nucleate spore. Probably vacuole functions in spore ejection.

FIG. 34. Mature four-nucleate conidiospore with slightly thickened wall.

FIG. 35. Spore germination.

FIG. 36. Spore germination. Three nuclei in germ tube which is penetrating insect wall.

FIG. 37. Fusion of hyphal bodies in preparation for zygospore formation. Two nuclei have migrated into conjugation tube.



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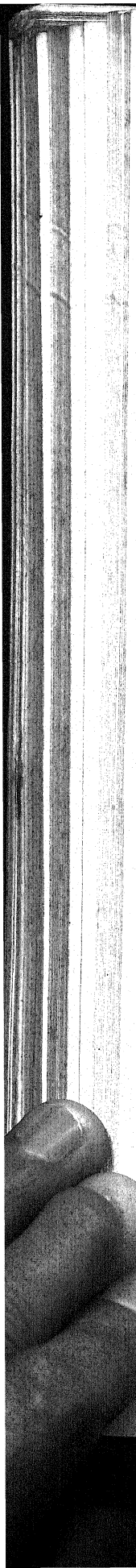

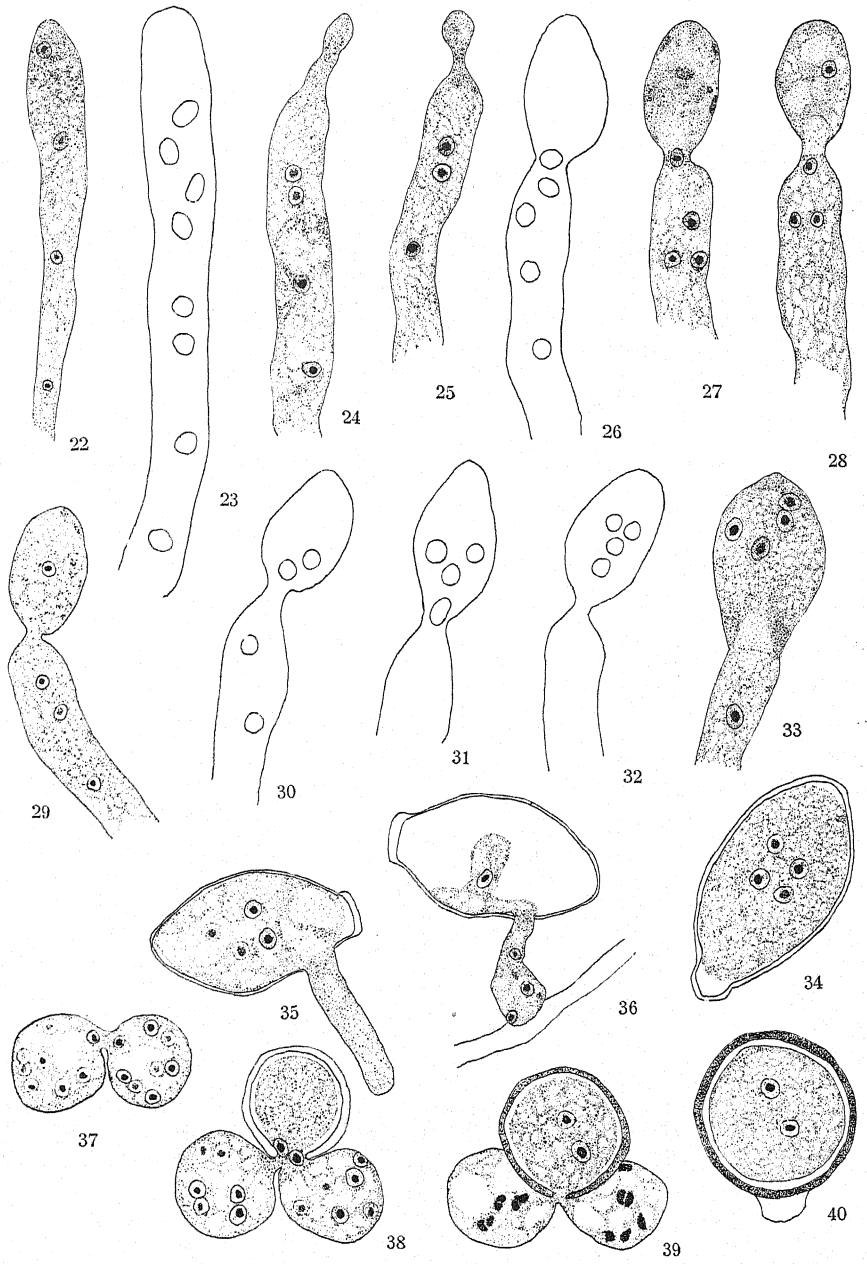

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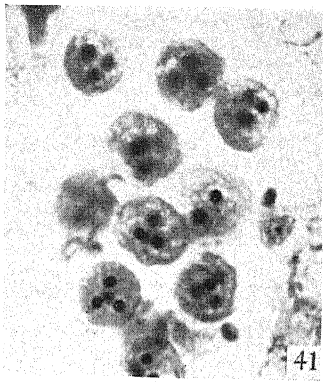

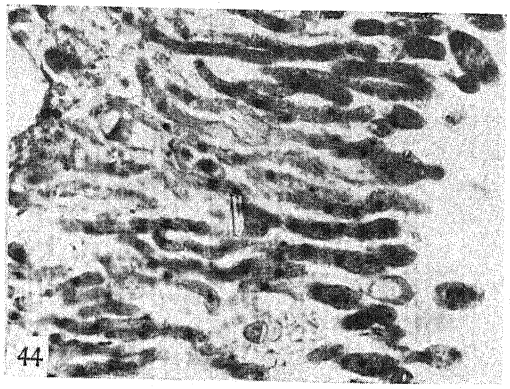

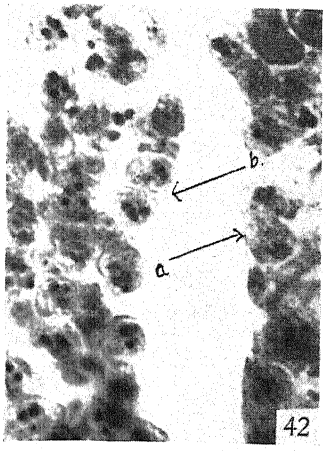

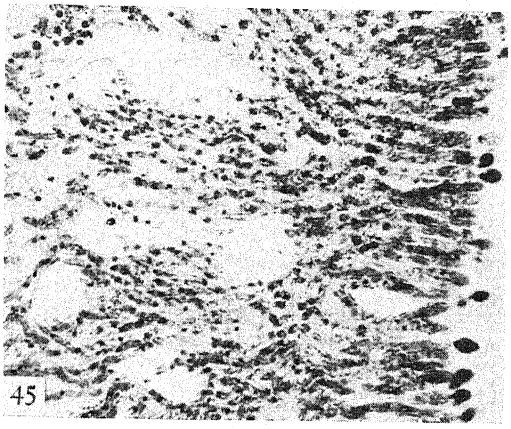

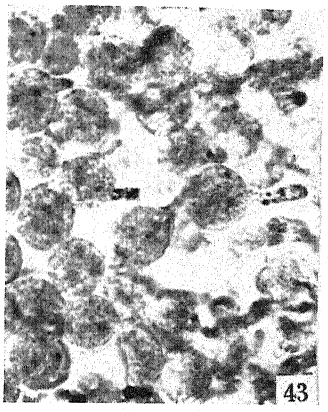

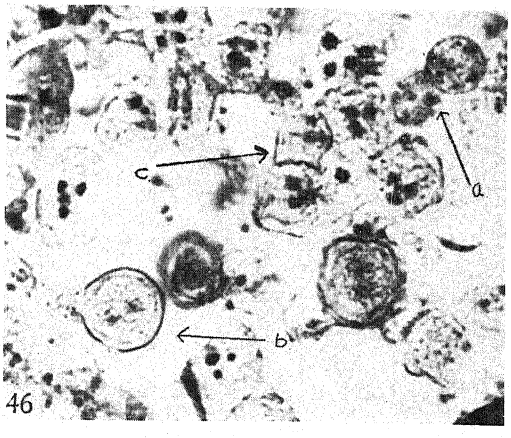

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FIG. 38. Zygospore formed due to fusion of contents of hyphal bodies, showing two nuclei in process of migration into maturing zygospores

FIG. 39. Thickened walls almost separating the zygospore from hyphal body. Nuclei of hyphal bodies in various stages of degeneration.

FIG. 40. Mature thick walled two-nucleate zygospore with remains of conjugation tube.

PLATE XVI

FIG. 41. Scattered hyphal bodies in early infection.

FIG. 42. Three hyphal bodies in stages of division. *a*, see FIG. 5. *b*, see FIG. 9.

FIG. 43. Hyphal bodies germinating to form hyphal tube as shown in FIGS. 11–19.

FIG. 44. Conidiophores and conidiospores of different ages.

FIG. 45. Conidiophores bearing spores arising from hyphal bodies.

FIG. 46. *a*, eight-nucleate gametes fusing (not median section). *b*, matured thick-walled spore with two nuclei in center. *c*, scattered hyphal bodies.

ORIGIN OF THE INTERCELLULAR SPACES IN *PEDIASTRUM*

ILLO HEIN

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In my studies on the mycelium of fungi (1928, 1930*a*, 1930*b*, 1930*c*) I have summarized the literature relating to the special problems concerning the form-controlling factors involved in their growth and have attempted to analyze the intercellular environmental relationships and other form-regulating factors concerned in the modifications which occur during development of the inherently filamentous hyphae into such structures as ascocarps, basidiocarps, and sclerotia. In the present study an attempt will be made to analyze the form changes of globular spheres under controlled conditions of tension and pressure and the case of more or less isodiametric cells in tissue continuity with the object of determining the mechanical forces which come into play in such systems. The behavior of the rubber spheres in the experiments suggest a possible analogy with the processes involved in the formation of the familiar four-lobed outlines, the spines, and intercellular spaces of *Pediastrum* species.

LITERATURE

In studies on *Pediastrum* Harper (1916, 1918*a*, 1918*b*) has given evidence that the four-lobed form of the cells with two long and two shorter arms is an inherited characteristic but suggests that it may have arisen and become "fixed in heredity" in sixteen-celled ancestors similar to *Pediastrum boryanum*. In such forms the cells may have been, according to Harper, surface tension globules whose spatial relationships became adjusted in the interplay of the principles of least surface and of bi-partition, assuming that they were held together as compactly as possible by adhesion in one plane. The form of the cells so spaced is then further modified by contact, pressure, and adhesion forces. The intercellular spaces found in the species regarded by Harper as the more specialized arise as the result of the four-lobed characteristic and their number and position are determined by the spatial interrelationships resulting from the incompatibility between the two principles mentioned.

OBSERVATIONS AND EXPERIMENTS

As possibly bearing on the problems as to the form and position of cells in masses, the number and shapes of their contact faces, and the origin and form of intercellular spaces I have made numerous experiments in which hollow-rubber and sponge-rubber balls, ping-pong balls, clay balls,

gelatine balls, and marbles were subjected to various conditions including the application of pressure from one, several, and all directions. I shall describe here the results of experiments with hollow rubber balls and discuss the peculiar dimples and invaginations obtained when they are enclosed and expand in limited space.

The hollow balls used were about $3\frac{1}{2}$ centimeters in diameter and expanded to nearly twice that diameter when immersed in xylol for a day or two. A balloon flask (5000 cc.) was filled with about forty such balls, no attempt being made to stack them in any particular fashion. Xylol was then added until all remaining space was filled by it.

The balls expanded until all their contacts became flattened so that by the following day a mass of polyhedra was obtained. The outside balls were flattened against the walls of the flask and exhibited from four- to six-sided faces whose margins met in trihedral angles (Pl. XVII, fig. 1; Pl. XVIII, fig. 10). As was to be expected, not all space was completely taken up by the expanded rubber even after the balls had attained the maximum possible expansion under the existing conditions. There remained elongated angular spaces at the intersections of the surfaces, in continuity with octahedral spaces which were triangular in section and similar to those found in parenchyma tissue. The interspaces form a connecting system throughout the mass and are usually triangular in cross section. The form of the spaces is shown in a model (fig. 9) which represents a part of such a system of spaces. Any three balls in the mass will enclose a space which is in form an elongated triangular prism with concave sides and continuous at each end with octahedral spaces whose alternate faces are connected with three similar triangularly prismatic spaces. The octahedral spaces consist of four triangular concave faces alternating with the four triangular faces which would be formed if the elongated prismatic spaces were cut off at the points where the two types of faces meet. All interspaces in the massed balls in the early stages of expansion may therefore be divided in two groups in respect to form. They are ideally either triangular prisms or regular octahedra, both with concave sides.

The massed balls when further expanded enclose other interspaces which are not connected with the spaces described but originate between adjacent faces. The faces of the outer balls against the flask become dimpled, or invaginate. The invaginations begin as very slight circular indentations in the center of the face and gradually become deeper and wider, often involving the whole surface (Pl. XVII, figs. 1, 2). When the indentations approach their maximum size they tend to be influenced by the general outline of the face and become roughly oval, quadrilateral, or irregular in outline (fig. 1). Some of the more deeply indented faces develop into an elongated trench-like space which in some cases results in the rubber breaking in a straight line at the base of the trench (fig. 1). Not all the contact faces showed dimples and even in the later stages of expansion

the balls were very variable in respect to the depth of the indentations formed. Doubtless owing to a lack of uniformity in thickness, porosity, and general quality of the rubber the rate of penetration of the xylol affects the amount ultimately imbibed.

In order to retain the form of the polyhedral rubber bodies obtained so that they could be separated and preserved for further study the xylol was replaced with paraffin in a manner similar to the conventional method of imbedding organic tissues. The flask was then broken and the balls with their altered forms in rigid condition were separated, numbered, the faces lettered, and records made of the position, form, number, and shape of the contacts.

While most of the faces of the interior balls were invaginated, a number of them were flat and some, convex. Several combinations in the form of the adjacent contact faces were noted. In some cases there were two adjacent concave faces (Pl. XVIII, fig. 5); in others a flat face was adjacent to a concave face (fig. 6); and in still others a concave face was adjacent to a convex face (fig. 7). Often the invaginations were very deep and their bases cracked as in the case of the exterior balls mentioned. Frequently cracks also appeared at the ridges formed between two faces (figs. 9, 10). The three balls shown in figure 8 were photographed in the positions in which they were found in the mass and the ball on the left has a concave face adjacent to a convex face on the upper ball and a concave face adjacent to a flat face of the ball on the right, while the two balls to the right of the figure have concave faces adjacent to each other.

In tables 1 and 2 are given the number of contacts, the number of sides which each contact face had, and the form of the contact, whether concave, flat, or convex, for all the balls used in the experiment.

The external balls had an average number of 8.11 contacts while the interior balls had an average of 13.50. The average for all the balls was 9.04. It is obvious that an insufficiently large number of balls was used in the experiment to give statistical data as to the type of polyhedron which the balls tend to achieve and no conclusions are therefore attempted but since the interior balls had an average number of sides of more nearly 14 than 12 it is possible that the tetrakaidecahedron may be typical. Further data along these lines will be reported later.

The photographs (Pl. XVIII, figs. 5–10) show pairs of the balls in approximately the positions in which they were found in the mass but slightly separated from each other to show the forms of the adjacent contact faces. Figure 5 shows a pair of balls whose adjacent faces are invaginated in opposite directions. Few models of this kind were obtained. Obviously the inequalities in thickness and quality of the inert materials used would make the delicate balance of forces necessary to achieve such equal responses of very infrequent occurrence. Figure 6 shows another pair of which the one on the left has an incurved face which was adjacent to the flat face of

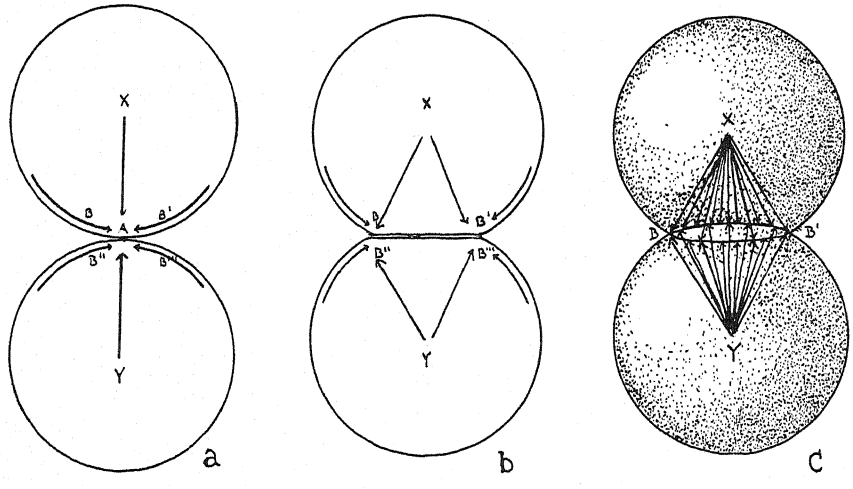
TABLE 1. *The Number of Faces and the Form of Each Face on the Exterior Balls and the Interior Balls*

			Flat faces with 3 sides	Invaginated faces with 3 sides	Evaginated faces with 3 sides	Flat faces with 4 sides	Invaginated faces with 4 sides	Evaginated faces with 4 sides	Flat faces with 5 sides	Invaginated faces with 5 sides	Evaginated faces with 5 sides	Flat faces with 6 sides	Invaginated faces with 6 sides	Evaginated faces with 6 sides	Number of contact faces
Exterior Balls:															
Ball number	1	has	1		1	1		2	1	3					9
	2	"	1		1	1	2	1	1	1					8
	3	"				1		1	1		1	1	3		8
	4	"				1			3	2	1	1		1	9
	5	"					4		1	1	1	1		1	9
	6	"				2		1		2	1	1	1		8
	7	"	2				2			1	1		2		8
	8	"				1		2	2	1	1	1		1	9
	9	"				3	2		1	3			1		10
	10	"				3			3	2		1	1		10
	11	"			2			1	2	1		2			8
	12	"					3			3		1	1		8
	13	"				1	2		2	1		1	1		8
	14	"				2	1				1	2	2		8
	15	"				1	2			1	2	1			7
	16	"				1	1		3	1		2	1		9
	17	"			1	2			2	3	1				9
	18	"				3	1	1		1		2		1	9
	19	"				4		1	1	1			2		9
	20	"				1	1			1	2		1		6
	21	"				1			2		2		2		7
	22	"				3	1	1				1	1		7
	23	"				3	1		3			1			8
	24	"				1				2	1	1	2		7
	25	"				1			3	2		1	1		8
	26	"				3			1	1		1	1		7
	27	"				3	1			1	1	2	1		9
	28	"				3		2			1	1			7
	29	"				2		2		2		1	1		8
	30	"				2		2		1	1	1	1		8
	31	"				2		1		2		2			7
	32	"				2		1	1	2	1	1			8
	33	"				2	1	1		1		1			6
	34	"				1		1		2	2	1	2		9
	35	"				2		2		2		1	2		9
	36	"				1		2		1	1	1	2		8
Average number faces															8.11
Interior Balls:															
Ball number	1	has				5			1		1	4		3	14
	2	"				3		1	2	1	1	3		1	12
	3	"				5			2	1	1	2	2	2	15
	4	"				4		1	3			3	3	1	15
	5	"				4			2		2	1	2		11
	6	"				5		1			1	3	3		13
	7	"				2		1	2		2		4	1	12
	8	"				4		2	3			2	4		15
Average number of faces															13.5

the ball on the right. In the two balls represented in figure 7 the face of the one on the right has evaginated, following into the space left by the adjacent invaginated face of the ball on the left. The mutually opposite pressure of the expanding balls, because of the irregularities in thickness and quality mentioned, are unequal and while undoubtedly the tendency of both adjacent faces was in the beginning to push outward when no resistance was offered, a strongly outwardly expanding face would tend to press inward an adjacent less strongly expanding one and we should have the condition represented in figure 7. Figure 4 is a median plane through all the balls and shows them in the forms which they assumed. Many interrelations are here represented but none appears to be typical.

For the analysis of the results of such an experiment we may best begin with the case of two hollow spheres, first in contact and then flattening against each other. We may next consider the case of a system of such hollow spheres lying in a single plane, and finally, the case of a system of such spheres which are stacked in various ways as are the balls in the globe described.

The balls used in the experiment as noted were not uniform in quality



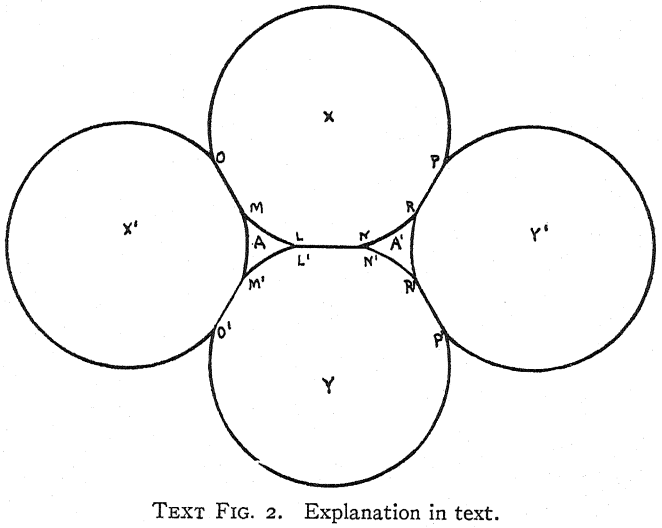
TEXT FIG. 1. Explanation in text.

and thickness of the rubber of which they are composed. Such uniformity, however, will be assumed for purposes of analysis. The balls are also assumed to expand in diameter in this case through tangential enlargement of their walls and not through inflation. Each ball has an opening so that it becomes filled with the surrounding medium and, as noted, the interior balls may be assumed to be under somewhat similar conditions of pressure.

Since at any point on the surface of the expanding spheres the forces of tangential expansion are, if we assume the uniformity postulated, equal in all directions, then flattening at any point on the sphere (text fig. 1*a*)

must overcome such tangential forces at the point of pressure. The resultant of such a system of forces is in a radial line through the point of contact, A. Two spheres in contact with each other will meet in a point and if at that point the spheres are exerting mutually opposite, radial pressures the resultant will be a straight line, XY in text figure 1*a*. Through tangential expansion of the walls the diameters of the balls are, as stated, increased and we may assume additional points of contact arranged in a circle about the original point of contact (text fig. 1*b*). We then have contact planes which are in the beginning circular in outline. Later, as the circular contact areas enlarge through the further expansion of the balls in the mass they assume various patterns as they meet with similarly expanding faces of adjacent balls. When viewed in section any two spheres with flattened contact areas will have two points, B and B' (text fig. 1*b*), at which the resultants of the forces of expansion meet with similar opposing forces B'' and B''' of the adjacent ball. These points represent in section the margins of the circular contact areas. When the circular contact planes are established they form the base of a cone of radial forces in each ball (BXB', and BYB', text fig. 1*c*).

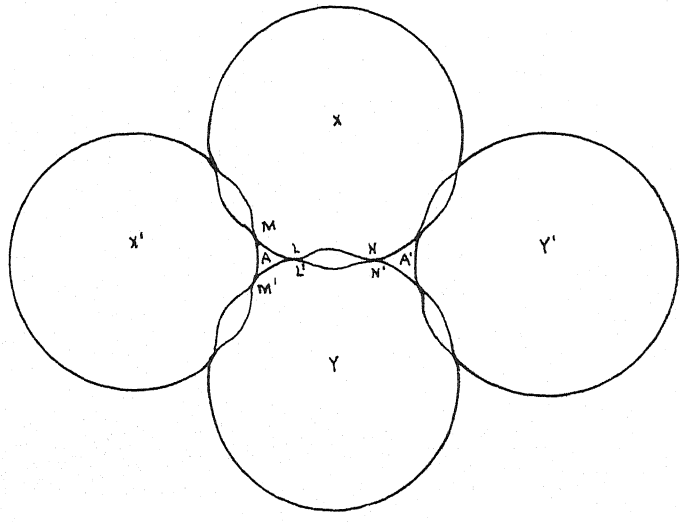
If we extend the analysis to a system of equal spheres lying in the same plane we find that when two such adjacent faces between any two spheres



TEXT FIG. 2. Explanation in text.

in a system of equal spheres (X and Y, text fig. 2) expand until the resultants of the forces of expansion are met at the margins by the opposing forces of similarly expanding spheres X' and Y', at the meeting points the forces if the spheres are equal will be in equilibrium. These two opposite points of equilibrium at A and A' in figure 2, transmitted at L and L', and N and N' to the adjacent contact faces LN and $L'N'$, limit the tangential expansion of these faces.

In order to simplify the analysis of the mechanical forces controlling the form changes of the adjacent faces of contact of the expanding spheres we may first consider the case of the forces in one plane projected through the centers of any pair of spheres (text fig. 2). Such a plane will show a system of pairs of straight lines of equal length, the lines OM, $O'M'$, LM, $L'M'$, PR, and $P'R'$, representing adjacent flattened faces in median section, which are connected at their ends with arcs MM, ML, $M'L'$, NR, $N'R'$, RR', and RN, which form the sides of triangles with concave sides. The triangles represent in sections the octahedral spaces described as occurring between stacked spheres. As the circles become wider the lines become longer and the arcs shorter. Since a long line offers less resistance to stress applied in its long axis than a shorter one, it follows that the longer one is the weaker and the longer it becomes the weaker it becomes. Furthermore, the system of arcs as described forms a triangular truss ML, $M'L'$ whose resistance to the lengthing line (broadening face) LN, $L'N'$, from which the stress is applied, becomes greater because its sides, the arcs, become shorter as the straight lines become longer. The long, straight, weak lines are undoubtedly supported by the expansion force thus giving them rigidity up to a certain point in their period of extension. The arcs continue to become shorter and more resistant, reducing the interspaces between their convex sides until the balance between strains and stresses in both arcs and lines can no longer be maintained and either the space between the



TEXT FIG. 3. Explanation in text.

arcs will become completely filled or the lines will bend as shown in text figure 3. Here the lines are bent away from each other. The figure represents a similar median section to the one shown in figure 2 but is at a stage when the balls have expanded somewhat further. The curved line

LN represents a section through an invaginated face of ball X, $L'N'$ of ball Y, and so on. In the material at hand the triangular spaces mentioned never become completely filled for the lines (representing the faces in section) bend some time before this is achieved.

If there are mutually opposite resistances between two adjacent faces LN and $L'N'$ in text figure 2, both will bend in opposite directions as in text figure 3, LN, $L'N'$. Inequalities in thickness or composition of the faces may result in both bending in the same direction and we have the condition represented in figure 5, Plate XVIII. The irregularities in the quality of the rubber may result in both faces remaining flattened up to a certain point and then one invaginates while the adjacent one remains plane as in figure 6, Plate XVIII. Various modifications of these combinations, too irregular and complex to be described, were obtained but with these we need not at the moment be concerned.

Some of the form changes which took place in the balls of the experiment are in appearance suggestively like that of the cells in certain *Pediastrum* colonies and possibly a similar explanation of the origin of their intercellular spaces may by analogy be found in the mechanical forces just described for the balls.

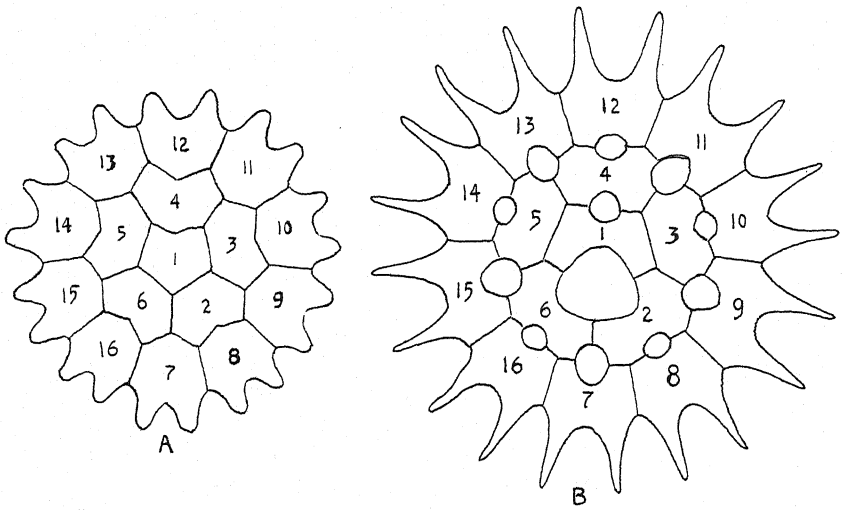
Harper has suggested that the inherited, four-lobed characteristic of *Pediastrum* cells is the result of intercellular adjustments in the colony involving contact, pressure, adhesion, and bipartition and that the forms so achieved have become fixed in heredity. Possibly the intercellular spaces in *Pediastrum* have originated as a result of mechanical forces analogous to those described for the balls and have similarly become fixed in heredity.

In order to carry over the analogy from the balls in the experiment to a *Pediastrum* colony, we may assume that the cells enlarge tangentially in the outer cell layers under pressure resulting from growth with adhesion. There is reason to assume that this has occurred, since in deeply lobed forms, *Pediastrum asperum* and *P. clathratum*, the ratio of surface area to volume is obviously greater than in the less deeply lobed forms like *P. boryanum*, and if in evolution the former arose from forms similar to the latter some such change may have taken place. For the present study it will be assumed that tangential enlargement of the outer cell layers has occurred under pressure resulting from growth with adhesion. With this assumption we have a condition comparable to that of the balls in the experiment.

If the mechanical forces which initiate the intercellular spaces in *Pediastrum* colonies are similar to those which initiate the spaces between the balls as described there should be in the cells a tendency to form spaces beginning at the centers of adjacent flattened faces. From the general appearance of the intercellular spaces between *Pediastrum* cells it appears that this may have occurred. If the sixteen-celled forms with intercellular spaces arose in evolution from those without them we may compare *P.*

boryanum (text fig. 4*A*) which has no intercellular spaces and *P. clathratum* (fig. 4*B*) which has spaces between adjacent cell walls, to two groups of similarly arranged balls of which those of one group have expanded under pressure and produced interspaces as described.

We may begin with an analysis of the intercellular spaces which occur in *Pediastrum* between adjacent tangential walls, which are comparable in the balls to such spaces as are represented in text figure 2. The origin of the spaces in each case may involve similar mechanical forces and we may apply the analysis given above for the balls to the cells of *Pediastrum* by comparing cells 1 and 4 (text fig. 4*A*) with *X* and *Y* (fig. 2) and cells 3 and 5 (fig. 4*A*) with *X'* and *Y'* (fig. 2). Accordingly, the adjacent walls between cells 1 and 4 expand tangentially as postulated above against the "barriers" made by cells 3 and 5 and become subject to pressures applied at the margins and in the direction of the long axes of the walls. Because



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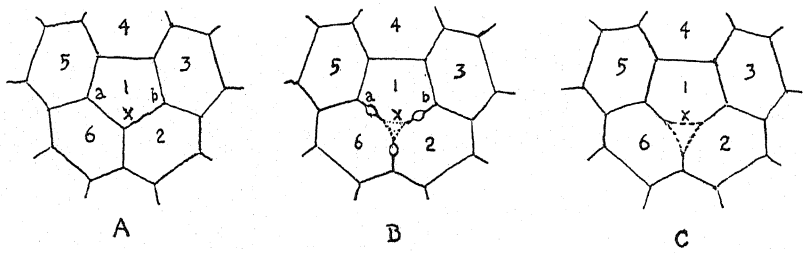

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TEXT FIGS. 4 AND 5. Explanation in text.

of the barriers (cells 3 and 5) the cell walls are incapable of further extension in tangential directions and the bending inward of the walls may then arise as a result of the mechanical forces similar to those described in connection with figures 3 and 4.

The forces which I have suggested as connected with the initiation of the spaces may not have brought about any considerable indentation in the walls but possibly amounted to nothing more than a slight separation of them. The enlargement of this separation to form the intercellular spaces may then be the result of the extension of the lobes through functional hypertrophy.

The initiation of the intercellular spaces between cells 1 and 5, and 1 and 3, which are in like positions with respect to adjacent cell walls, may be similarly explained and likewise the spaces between cells 2 and 8, 3 and 10, 4 and 12, 5 and 14, and 6 and 16 of the second and third series of cells (text fig. 4*B*).

The large triangular intercellular space between cell 1 and cells 2 and 6 in text figure 4*B* appears as if it may have resulted from the fusion of two or possibly three spaces. Possibly in this case there are initiated between the adjacent cell walls of cells 2 and 6, 2 and 1, and 1 and 6 in text figure 5*A* (detail of fig. 4*B*) intercellular spaces, in the same manner and as a result of similar forces to those described above for cells 1 and 4, and so on. But here the inherently bi-lobed tendency established in the cells causing an extension of the two arms to be formed at *a* and *b* (cell 1, fig. 5*B*) will tend to apply tension to the cell walls *ax* and *xb*, straighten out the angle at *x* and thus initiate another space at *x*, or enlarge the minute intercellular space which may already exist. This process may continue until all four spaces become confluent and form a large triangular space shown in text figure 5*C* at *x*. The transformation of this space to the typical rounded form found in *Pediastrum* may then be a matter of surface or form tensions. The spaces formed at the trihedral angles made between cells 2, 7, and 6; 5, 6, and 15; 4, 5, and 13; 3, 4, and 11; and 2, 3, and 9, may have originated in a like manner.

Summary

A balloon flask was filled with hollow toy rubber balls which expanded to nearly twice their diameter when immersed in xylol and resulted in the formation of characteristic indentations in the centers of the contact faces. The balls were imbedded in the expanded condition with paraffin, the flask was then broken, and the balls separated for study.

The interior balls had an average number of 13.5 contacts while the exterior balls had an average of 8.11. The contact faces had from 3 to 6 sides and were either flat, convex, or concave. In some cases the adjacent contact faces were both plane, in others a concave face was adjacent to a convex face, and in still others the adjacent faces were both concave.

An analysis of the mechanical factors involved in the formation of the indentations obtained is given first for a pair of balls and then for a system of balls lying in a single plane.

In the cases in which the adjacent contact faces were both concave, lens shaped spaces were formed between them. The appearance of these spaces suggests a possible analogy with the intercellular spaces in certain *Pediastrum* colonies. An attempt is made to analyze the formation of the intercellular spaces in *Pediastrum* on the basis of the analogy with the spaces formed between the balls used in the experiment.

The writer feels it a pleasure to record his indebtedness to Dr. R. A. Harper for his stimulating criticisms.

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DESCRIPTION OF PLATES

PLATE XVII

FIG. 1. A side view of the flask containing the expanded rubber balls imbedded in paraffin. Most of the balls visible in this view show the typical invaginations obtained.

FIG. 2. Opposite side of the flask. This photograph was taken before the balls had expanded completely.

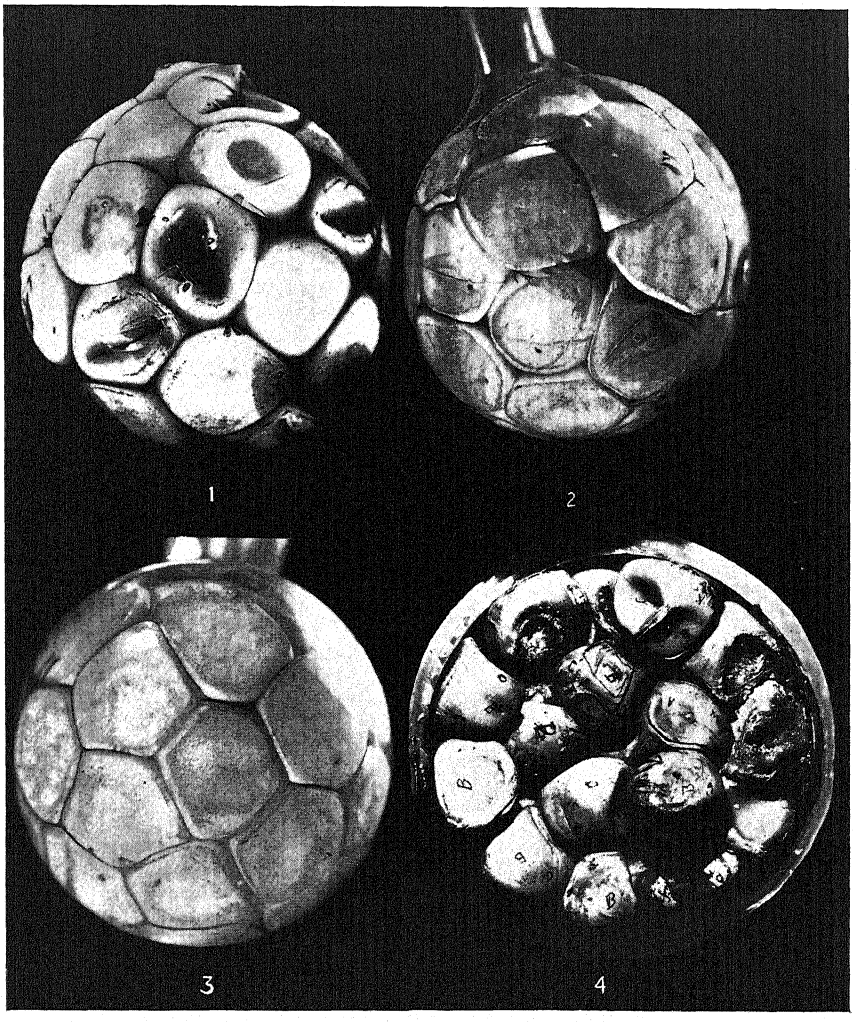
FIG. 3. The view is taken from the bottom and shows the characteristic configuration of the faces formed against the wall of the flask. The photograph was taken when the balls had expanded to the maximum but no invaginations occurred in this part of the flask.

FIG. 4. A median plane through the mass. Invaginated, evaginated, and flat faces are shown.

PLATE XVIII

FIG. 5. Two balls taken from the mass and placed in the positions in which they were found but separated slightly to show the adjacent invaginated faces.

FIG. 6. A pair of balls of which the one on the left has an invaginated face which was adjacent to the flat face of the ball on the right.



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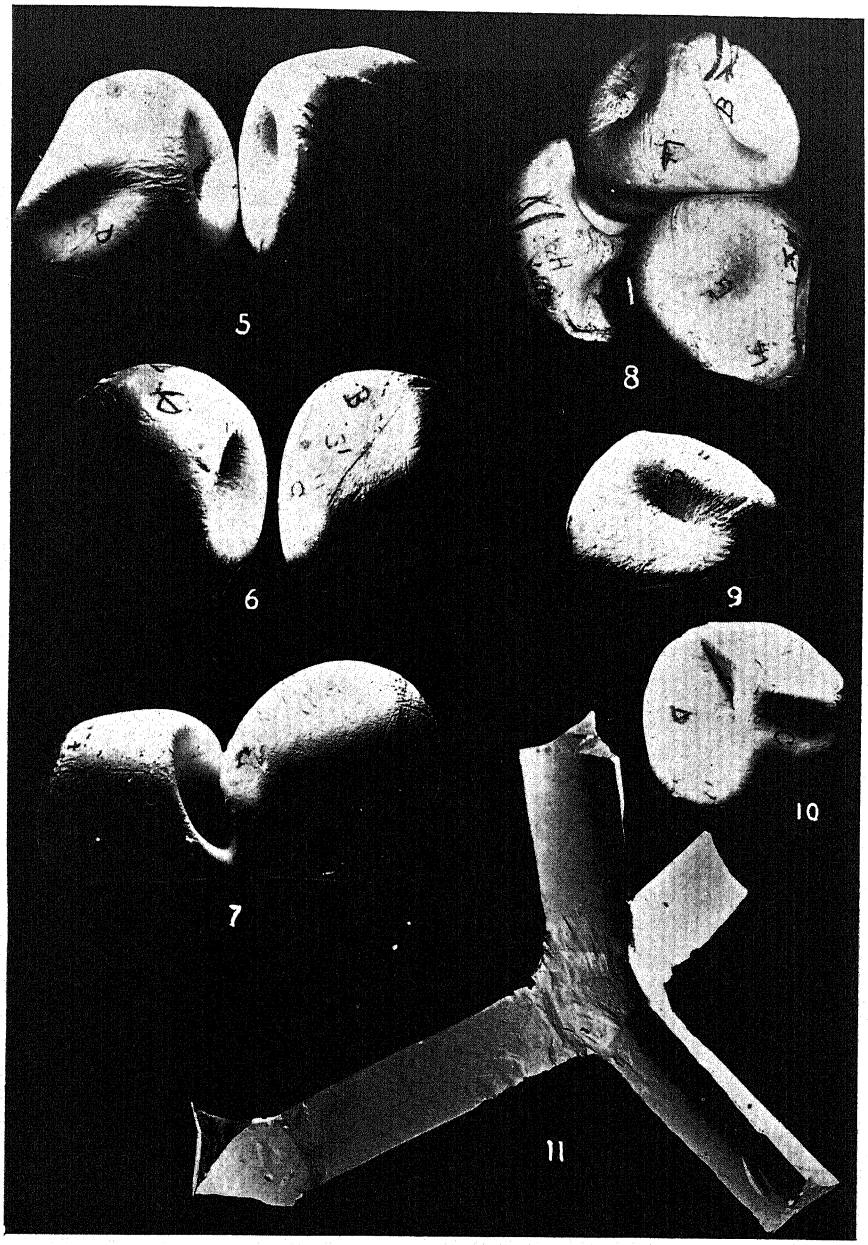


HEIN: PEDIASTRUM

FIG. 7. The ball on the left has an invaginated face which was adjacent to the evaginated face of the ball on the right.

FIG. 8. Three balls taken from the mass and placed in the positions in which they occurred. Various combinations of the form of the adjacent faces are represented (see text).

FIGS. 9 AND 10. The ridges formed at the interfacial angles between two invaginated faces.

FIG. 11. Model of a part of the system of interspaces formed between the balls, showing the characteristic triangular prismatic form of the spaces with concave sides. Each of these spaces is continuous with an octahedral space at each end.

THE EMBRYOGENY OF *CHAMAECYPARIS OBTUSA*

J. T. BUCHHOLZ

(Received for publication September 22, 1931)

There is very little literature bearing on the reproductive morphology of *Chamaecyparis*. Our present knowledge of this form is largely based on inference from the morphological investigations which have been reported for other Cupressineae: *Thuja* (8, 20), *Biota* (3, 9, 20), *Libocedrus* (2, 9), *Juniperus* (7, 10, 11, 12, 19, 20), and *Cupressus* (21). As far as the embryogeny is concerned, there are at least two types known for Cupressineae. Strasburger (20) and more particularly Land (8) described an embryogeny for *Thuja* in which cleavage polyembryony does not occur. The writer (3) found cleavage polyembryony a very characteristic feature of *Biota*, a supposedly closely related form. On the other hand, a number of other species of *Thuja* have been dissected: *T. plicata*, *T. Standishii*, and several varieties of *T. occidentalis*, all of which agree with the observations of Land in regard to their simple polyembryony.

It was found and pointed out elsewhere (3) that *Biota orientalis*, called *Thuja orientalis* in current manuals, is not a *Thuja* at all. Its embryogeny differs from this genus much more than it differs from *Libocedrus* or even *Juniperus*. When one considers and compares the other morphological features of these two genera, there is very little aside from the general characters of the sub-family which they have in common. The taxonomic resemblances suggesting generic identity are largely superficial. It is surprising to find that these genera have ever been merged, and there is no doubt of the validity of the genus *Biota* of Endlicher. It is evident that there are at least two types of embryogeny among Cupressineae and it becomes a matter of interest to discover which type is found in any particular genus of this sub-family.

Many species of *Chamaecyparis* have very small cones and ovules, a feature which renders dissection of embryos difficult. Some years ago I had occasion to dissect some material of *C. thujoides* in which the embryos were slightly older than the stages desired. All that could be determined with certainty in these small ovules was the fact that cleavage polyembryony is found in this genus and that some of the embryos showed an apical cell. During the summer of 1927, a few cones of *C. obtusa* were obtained from the plantings on one of the estates near Cold Spring Harbor, N. Y. Here both the ovules and the embryos were larger and could be dissected and handled without much difficulty. The accompanying drawings were prepared from some of these dissections.

In the Cupressineae, where the archegonia are grouped into an arche-

gonial complex, several eggs may be fertilized by the same pollen tube (19). Nearly all of the dissections showed that two or more neighboring eggs had been fertilized and the embryo complexes which were drawn were composed of two or more embryo systems. The general technique followed in the preparation of slides has been described elsewhere (3).

Text figure 1 shows an embryo complex which had been pulled apart in dissection. The cap shown on the top, covering the upper portions of the two suspensor systems, is a small portion of the female gametophyte. The elongated cells projecting from this structure are prosuspensors (*ps*), nearly all of which have themselves become embryonic unless they have collapsed. The six greatly elongated cells are the primary suspensors (*s*) belonging to the embryos which are found below at their tips. The embryonic cells at the lower end are, for the most part, still one-celled, though two of them have become three-celled. There may have been a few units lost in dissection, and probably a few of the strands found among the prosuspensors above belong with the embryos below.

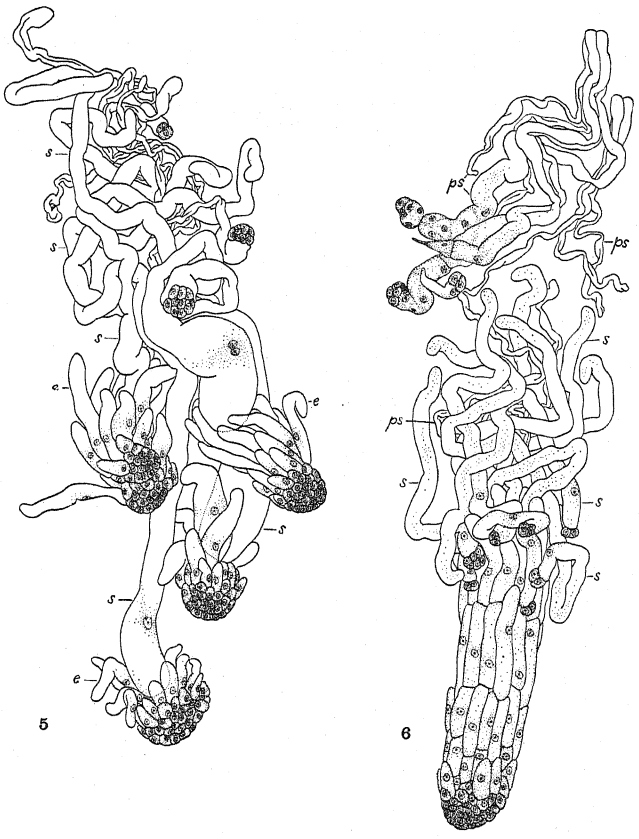
Text figure 2 represents a slightly older stage in which a more intimate association is found between the prosuspensor strands above and the primary suspensors of the lower embryos. The difference in the stage of development between figures 1 and 2 is not great, and is reflected very largely in the longer primary suspensor of the lower group of embryos.

Prosuspensor cells come from an upper layer of embryo initials and are the first cells of the proembryo to elongate. These thrust a group of lower embryo initials deeper into the prothallium and there the latter divide, each cutting off an embryonic cell from a primary suspensor cell. The primary suspensor cells elongate greatly, imbedding the embryonic cells deeply in the gametophyte, and this elongation also crowds the prosuspensor cells back toward the micropylar end of the embryonic cavity which has been formed by digestion of the central portion of the female gametophyte. The prosuspensor strands are, therefore, slightly different in origin from the primary suspensors, and being embryo initials they may themselves proliferate to produce embryos if they do not expend all of their activity as elongating cells. However, these embryos always appear deformed when compared with the normal embryos below. Sometimes the prosuspensor cells collapse. They may, in fact, shrivel so completely that they are difficult to find. Figures 1 and 2 show some of the collapsed strands of prosuspensor cells and both figures have many of the misshapen embryos which result from the proliferation of these upper embryonic elements. Prosuspensor cells may become only multinucleate and enlarged, they may become filled with cellular tissue, or cut off cells near the tips.

Text figures 3 and 4 show some later stages of the lower embryos borne on primary suspensors. The embryos have now become multicellular. In these stages, it appears likely that the embryo develops by apical cell growth, but the apical cell could not always be identified with certainty.

TEXT FIGS. 1–4. FIG. 1. Embryo complex of *Chamaecyparis obtusa*, showing upper portion covered by a cap of gametophytic tissue with prosuspensors (*ps*), many of which have become embryonic and lower portion consisting of primary suspensors *s* belonging to the embryos below. × 50. FIG. 2. Embryo complex of *Chamaecyparis obtusa*, slightly older than figure 1, with prosuspensors and primary suspensor still connected. Details lettered as in figure 1. × 50. FIGS. 3 AND 4. Stages of individual embryos borne on primary suspensors *s* at the time of beginning of embryonal tubes *e* which form the secondary suspensor. × 50.

Figure 4 is in a slightly later stage than figure 3 and is beginning to form a fringe of elongated embryonal tubes which project back on all sides enveloping the primary suspensor. The individual embryos below in text figure 5 show these embryonal tubes somewhat more developed and this embryo complex illustrates how the primary suspensor cells may sometimes become enlarged at their lower ends, also how the upper ends of many of the primary suspensors may collapse. The prosuspensor portion of this embryo complex

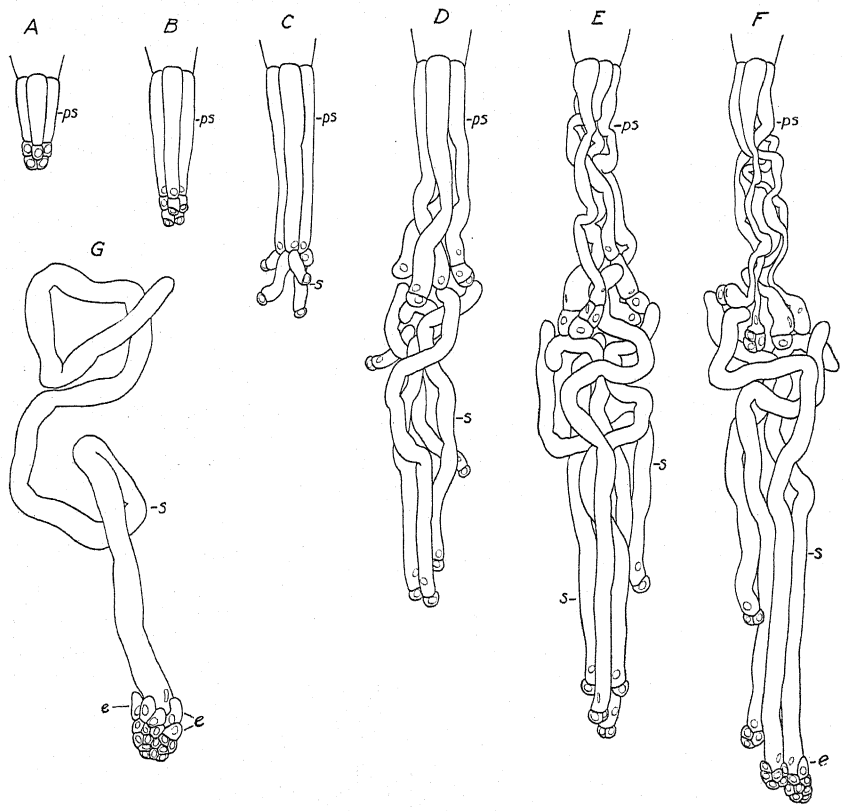


TEXT FIGS. 5 AND 6. FIG. 5. Lower portion of embryo complex consisting of 7 multicellular embryos attached to the enlarged ends of primary suspensors. As the secondary suspensors are formed, the embryonal tubes *e* surround the ends of primary suspensors. × 50. FIG. 6. Embryo complex made up of two embryo systems. Large terminal embryo has well developed secondary suspensor and many small embryos still on the primary suspensor, with prosuspensors *ps* above. Some of the prosuspensors at the left have become embryonic, those at the right have collapsed. × 50.

is not shown. The older embryos are now producing massive secondary suspensors of embryonal tubes. Some of the embryos of figure 5 still have apical cell growth, but the apical cell cannot always be recognized with certainty, and is not easily shown in drawings of the surface views.

They become more distinctly recognizable in the views of a deeper, more median plane of focus, if the preparations are not stained too deeply.

Text figure 6 is an embryo complex in which one of the embryos has become very large in comparison with the others. It is now a well developed embryo from which a massive secondary suspensor is developing while successive layers of embryonal tubes elongate from the cells of the distal end of the embryo. This is the oldest stage which was observed, but represents a period of development in which the various parts of the embryo (stem tip, root tip, and cotyledons) are still undifferentiated. Many of the prosuspensor strands above have collapsed; a few which have developed into misshapen embryos are still recognizable.



TEXT FIG. 7. Diagrammatic representation of successive steps *A*–*F* in the development of a single embryo system (single zygote). *G* represents a single primary embryo of slightly older stage; *ps*, prosuspensor cells; *x*, primary suspensors; *e*, embryonal tubes forming secondary suspensor.

Text figure 7 is a series of diagrammatic sketches representing the events in the development of a single zygote or embryo system of *Chamaecyparis*. Stages *A* and *B* were not actually observed, but are supplied by

inference from a knowledge of *Biota* with which the other stages are in fair agreement. Parts of the stage shown in *C* could be identified in the dissected material. The other stages *D–F* are essentially the same as a single embryo system of the two or three zygotes found in figures 1, 2, 5, and 6 with the parts labeled, and *G* shows a single lower embryo unit after it has become multicellular, on the end of its primary suspensor (*s*).

It should be borne in mind that figure 7 is intended as a diagrammatic interpretation of the embryogeny, and that these are not camera drawings.

Discussion

Aside from several minor details the early embryogeny of *Chamaecyparis* it is general agreement with that of *Biota* (3), and it also resembles observed stages of *Libocedrus* (2) very closely. Cleavage polyembryony is found in all of these forms which probably represent the embryogeny typical of the Cupressineae.

There are some differences between the individual embryo units of *Chamaecyparis* and *Biota*. Those of *Biota* (3) pass through a stage (see fig. 11*a* of the work cited, in the same stage as our text fig. 1) in which the embryonic cells found on the ends of the primary suspensors have a relatively thick inner cell wall immediately around the protoplast, and this is surrounded in turn by a thin wall by which it is attached to the primary suspensor. The corresponding cell wall is not specially thickened in *Chamaecyparis*, and there seems to be a tendency for an earlier division to take place in this terminal embryonic cell. Likewise, *Chamaecyparis* has prosuspensor cells which proliferate as embryo units in slightly earlier stages.

There appears to be another difference between the embryos of *Chamaecyparis* and *Biota*, for the latter was found to give rise to additional embryos by a process of budding (3). Several lobes or bud-fragments may appear in a multicellular embryo of *Biota* at the stage when the primary suspensor has become fully elongated and may have collapsed, but before embryonal tubes begin to elongate. This budding phenomenon was not observed in *Chamaecyparis obtusa*, though it is possible that some form of budding may be found to occur on rare occasions.

The type of embryogeny found in *Thuja*, in which no cleavage polyembryony occurs, is different and is found, as far as known, only in the various species of *Thuja* and their varieties. Judging from the facts known about the proembryos (8, 9), the organization of the cells in the early divisions is very similar. It appears that in *Thuja* all of the embryo initials, save the one found at the tip, refrain from forming embryos. This one proliferates rapidly while the other cells elongate in turn to form a suspensor made up of several cells from the very beginning. If two cells remain at the tip rather than a single cell, the embryo may appear two-lobed for a time, as Land (8) has pointed out, but one lobe may outgrow the other without separation so that usually only one embryo results from each

fertilized egg. The compound nature of the embryo in Cupressineae is shown by all members of the group which have cleavage polyembryony, and may be inferred for *Thuja* if this type has been derived from forms with cleavage polyembryony, much as the *Picea* and *Pseudotsuga* type seems to have been derived from the *Pinus* type among Abietineae (1, 4).

Two kinds of embryos may be found in the same embryo system of *Chamaecyparis*, a lower group of primary embryos which become deeply imbedded in the prothallium, and an upper group of embryos coming from the cells of the prosuspensor. The primary embryos come from the group of embryo initials terminal to the prosuspensor.

The other kind of embryos present in the embryo system are those coming from the cells of the prosuspensor. These embryo initials begin their development by elongation, which may be followed by internal cell proliferation. Prosuspensor cells may be regarded as the morphological equivalents of the rosette cells of Abietineae. They are equivalent to rosettes with respect to their embryonic character as well as their position in the proembryo.

In another place (6) the writer is describing the suspensor and early embryo of *Cryptomeria*, one of the Taxodineae. While the proembryos of Cupressineae and Taxodineae have been described as similar, and although there are some outward resemblances between the two types of embryo complex, a careful examination shows an important difference in the embryogeny of these two groups. It appears that the Taxodineae usually have no primary suspensor. They have prosuspensors which are morphologically equivalent to the structures so named in *Chamaecyparis*, on the ends of which the embryo initials develop into multicellular embryos without the intervention of the very long primary suspensor. A massive secondary suspensor of embryonal tubes is formed around the end of the prosuspensor rather than the primary suspensor. The embryonal tubes may begin to elongate a few at a time rather than the fringe of many embryonal tubes, shown in text figures 4 and 5.

Sciadopitys (5) has cleavage polyembryony and a plan of embryogeny (aside from differences in the proembryo) which agrees more or less with that of *Chamaecyparis*. There may be a group of many rosette cells, a few scattered rosette cells, or no rosette cells at all. When no rosette cells are found, the embryogeny resembles that of *Chamaecyparis* most nearly, but since the prosuspensor cells elongate greatly and do not form embryos in *Sciadopitys*, there seems to be a difference in regard to this feature. The individual embryos of *Sciadopitys* have a well developed primary suspensor, but the terminal embryonic cell divides rapidly and without delay to form an embryo of several or a dozen cells before the primary suspensor has elongated greatly. *Sciadopitys* is a generalized type from which the embryogeny of both the Cupressineae and the Taxodineae may have been derived.

Saxton has made a series of investigations on the embryogeny of *Tetraclinis* (18), *Widderingtonia* (13, 15), *Callitris* (14), and *Actinostrobus* (16). The embryogeny of this group is distinctive and differs somewhat from that of Cupressineae. The archegonial complex is lateral in position and walls appear on the proembryo at an earlier stage. The zygotes all have cleavage polyembryony and show a condition which may have been derived from Cupressineae through some kind of reduction. The individual embryo units seem to have primary suspensors and Saxton also mentions for *Callitris* a period of inactivity in the embryonic cells at the tip of the suspensors which may correspond to this stage of relative inactivity of this cell in *Biota*. Possibly the prosuspensors are parts which are suppressed in *Callitris* and *Actinostrobus*. Saxton proposes the inclusion of three of these four genera in a separate group Callitroidae (17, 18), which seems to be fully justified, and is apparent on the basis of various features of the morphology aside from the embryogeny.

Saxton carried on his investigations by means of serial sections. It would be of considerable interest if someone should follow up his investigations by the use of dissection methods, since there are doubtless some features of the suspensor system which would be shown to better advantage.

Within the immediate group of Cupressineae, the embryogenies of *Chamaecyparis*, *Libocedrus*, and *Biota* are in substantial agreement. With respect to the minor differences which have been observed *Libocedrus* stands between *Chamaecyparis* and *Biota*, while all of these forms differ from *Thuja*.

Summary

1. The early embryogeny of *Chamaecyparis obtusa* is described, including stages which are important for an interpretation of the type of polyembryony.

2. *Chamaecyparis* has cleavage polyembryony which may result in two types of embryos: primary embryos, borne on primary suspensors, and secondary embryos which are formed from a proliferation of some of the prosuspensor cells.

3. The secondary embryos are probably the morphological equivalent of the rosette embryos found in Abietineae and certain other conifers.

4. The individual embryo units of *Chamaecyparis* become multicellular by apical cell growth.

5. The primary suspensor of *Chamaecyparis* is not succeeded by single-celled additions, but by a massive secondary suspensor composed of many embryonal tubes.

6. Differences between certain Taxodineae and Cupressineae in which the embryo systems may resemble each other superficially are discussed, showing that in Taxodineae the primary suspensor is usually absent.

7. The discussion outlines the probable steps in the evolution of the

various types of embryogeny thus far found in Cupressineae, Taxodineae, and other related groups.

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STRUCTURE AND DEVELOPMENT OF *ZONARIA FARLOWII*

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Morphological studies in the Dictyotaceae have been confined largely to *Dictyota*, whose life history has become well known through the work of a number of investigators. *Padina* has received some critical attention, but the dozen or more other genera have been largely neglected. One of these, *Zonaria*, is of particular interest because of the peculiarity of certain features of its morphology.

For the most part, *Zonaria* is a tropical genus, its various species being widely distributed. The present investigation is based on *Z. Farlowii* S. and G., which grows abundantly along the coast of southern California in the vicinity of Laguna Beach. Nearly all of the material used in the present study was collected near Abalone Point, about two miles northwest of the village. A few plants were collected along the coast about midway between San Pedro and Redondo Beach, but the species is much less abundant there. Other Dictyotaceae found at Laguna Beach include *Dictyota flabellata* (Collins) S. and G., *D. Binghamiae* J. Ag., and *Neurocarpus zonarioides* (Farlow) Howe, forms which are also common farther north. Setchell and Gardner (10) give the distribution of *Zonaria Farlowii* as "Santa Barbara to San Diego."

In the region where the material for the present study was collected, *Zonaria* grows attached to rocks in tidepools. It grows where, at the time of the lowest spring tides, it will still be covered with water. Lack of facilities for gathering plants in deep water or at night restricted collecting to times when the lowest tides came during the hours of daylight. The datum used by the U. S. Coast and Geodetic Survey in reckoning tidal heights along the California coast is mean lower low water (11). It was found that the best collecting could be done when the height of the water was at or below this level, and that little or no material could be obtained when the water was more than 15 cm. above it.

During the months of June, July, August, and September the lowest tides occur at night, and consequently daytime collecting in shallow water was further limited to the period of the year from October to May, inclusive, the most favorable spring tides occurring from October to February, the most favorable neap tides during March, April, and May. Since the facilities of a marine laboratory were not available, daily observations could not be made, or culture work carried on under natural conditions. The location of the writer's headquarters sixty miles from Laguna Beach,

and the pressure of other duties, made frequent trips to the source of material impossible. Twelve collecting trips were made during the period from February, 1929 to May, 1931.

Methods

The meristematic and reproductive cells of *Zonaria*, like those of many other brown algae, contain large quantities of a mucilaginous substance which gives trouble in preparing sections for study. Unless much of it is removed during fixation, the material becomes difficult to cut, to affix to the slide, and to stain properly. After trying a number of different formulas containing chromic and acetic acid in varying proportions, both with and without the addition of osmic acid, the following was found to be most satisfactory:

Chromic acid	1 g.
Glacial acetic acid	3 cc.
Osmic acid (1%)	1 cc.
Sea water	96 cc.

It was found that the proportion of acetic acid may be increased up to 5 cc. with satisfactory results, but if less than 3 cc. is used, the mucilage remains in the cells. In some formulas the osmic acid was increased up to 7 cc., but there was no apparent advantage, either in the rapidity of fixing or in the precision of staining, to compensate for the greater degree of blackening of the cell contents which results.

The material was fixed at the beach, left in the fixing solution for six hours, and then washed for twelve hours in many changes of sea water. After being placed in equal parts of sea and fresh water for an hour, it was transferred to fresh water for another hour, and then run through the usual series of alcohols and xylols. Paraffin sections were cut 4 μ in thickness, and stained in iron-alum hematoxylin, frequently with the addition of a light counterstain of erythrosin.

Habit

Each plant of *Zonaria Farlowii* consists of a fan-like cluster of thin, flat, branching fronds which form many overlapping parallel series, become stipe-like below, and are attached at the base by a stupose, disk-shaped holdfast (text fig. 1). The shape of the plant is due to the fact that growth of the fronds is terminal and that branching, which is profuse, occurs in but one plane. The plants become semi-circular in outline, or nearly so, and usually measure 6–10 cm. from the base to the outer margin, but occasionally reach a length of 15 cm. They stand vertically in the water, the distal margins of the fronds pointing upward. As a result, one frond does not overlie another, but all are held parallel to the light rays.

The individual fronds are light brown and flabellately divided. As a rule the segments reach a length of 8–15 mm. and a width of 5–10 mm.

before branching occurs, but considerable variation exists with respect to these dimensions. The holdfast, usually 30–40 mm. in diameter, is composed of a tangled mass of rhizoids. The older, stipe-like portions of the plant become somewhat costate by the abundant growth of rhizoids on both sides of the median line. As these arise, the lateral wings of the frond disappear. There is no thickening of the median portion of the older parts of the plant by cell division.

In sharp contrast to *Dictyota*, which grows by means of a single apical cell, the growth of *Zonaria*, like that of *Padina*, results from the activity

TEXT FIG. 1. *Zonaria Farlowii*, habit of plant. × 1.

of a marginal row of initials. Growth occurs chiefly in length, but to some extent in width as well, meristematic activity occurring at the rounded distal margin of the fronds. Branching results from the death of a few marginal cells. Further growth may be uniform on each side, as is usually the case, or the development of one or more branches may be retarded.

INTERNAL STRUCTURE OF THALLUS

The development of the thallus takes place by the cutting off, in one plane, of posterior segments from the apical row of initials. In a section cut parallel to the surface of the thallus, the apical cells appear long and narrow (Pl. XIX, fig. 7), but when cut in a plane at right angles to the surface and parallel to the direction of growth, each initial appears as wide as the mature thallus (fig. 5). Richards (7) studied the development of the thallus in *Zonaria variegata*. He found that, in each of the marginal cells, two unequal segments are formed—a larger distal cell which continues the elongation of the frond, and a smaller proximal one. The latter divides into three cells by the appearance of two walls parallel to the surface of

the frond, thus forming an inner cell between two outer ones. The inner cell then gives rise to one or two layers of cells above and below, the mature thallus consisting of 5–7 layers.

In *Zonaria Farlowii* the situation is essentially similar to that described above, except that the two cells resulting from the division of the apical cell are more nearly equal in size. Furthermore, following the cutting off of the epidermal layer on each side of the frond, the inner cell gives rise, by successive divisions parallel to the surface, to two, four, and finally six layers of cells (fig. 5). Thus the mature thallus consists, as a rule, of eight layers of cells. The central cells do not undergo additional divisions, but the superficial cells do, each primary epidermal cell most commonly forming four daughter cells having either a linear or a quadrilateral arrangement, or a combination of the two. Consequently, a single layer of four epidermal cells usually stands above and below each row of central cells, although often there may be a few more or less than four (figs. 5, 8, 9).

As seen from the surface, the longitudinal rows of cells converge toward the median portion of the thallus. As pointed out by Setchell and Gardner (10), the cells have the form of a parallelopiped. The epidermal and marginal cells have relatively dense cytoplasm and numerous chromatophores, while the inner cells are highly vacuolated and have few chromatophores. The nuclei in the vegetative cells are small, having a diameter of approximately 5 μ. The cell walls are thickened, probably with pectic substances, the middle lamella being distinct. The cell walls on both outer surfaces are two or three times as thick as those within the thallus. There are no intercellular spaces.

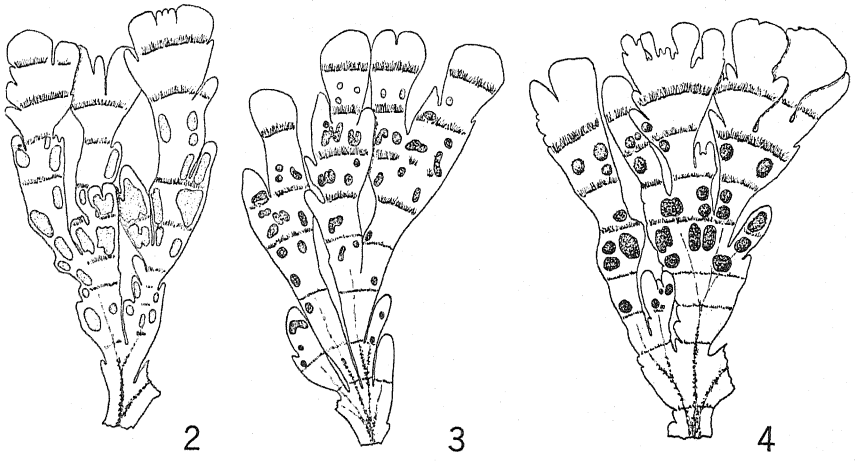
The superficial and marginal cells are rich in reserve food. This is mainly in the form of oil, since numerous black droplets remain in sections where the fixative used contained osmic acid, but are not present otherwise. The meristematic cells at the apical margin of the thallus have nuclei 15–20 μ in diameter. While ordinarily it was difficult to detect the presence of a centrosome or of astral rays in these cells, distinct radiations were apparent at the distal pole of the nucleus in material fixed in sea water containing 5 percent formalin and 3 percent glacial acetic acid, and stained in Delafield's hematoxylin (fig. 6). The presence of a centrosome could not be demonstrated, however, although it is probable that one is present. In *Dictyota* Mottier (6) found no aster or centrosome in the apical cell during the resting period, but "as soon as karyokinetic activity begins, an aster is always to be observed near the nucleus, even while the latter still presents the structure characteristic of the complete resting stage."

Rhizoids and Hairs

Although the plants of *Zonaria Farlowii* are attached to the substratum only at their base, short rhizoids are present on older parts of the thallus. They appear about 2 cm. behind the distal margin, arising on both sides of

the thallus along its median line (text figs. 2–4), but farther back, where the thallus is stipe-like, they cover it on all sides. The rhizoids are slender branching filaments, brown in color, and growing at their tips (Pl. XIX, fig. 11). Their cells are uninucleate, but differ in the density of their contents and in the number of chromatophores present.

Another kind of hair-like outgrowths is also generally present in *Zonaria Farlowii*. They are formed in localized concentric groups on the young parts of the fronds, each group occupying a narrow transverse band or



TEXT FIGS. 2–4. Male, female, and asexual fronds. × 2.

zone (text figs. 2–4). These sterile hairs may occur on both sides of the frond, but ordinarily they are much more abundant on one side than the other. The distance between successive bands of hairs is approximately 2–4 mm. On narrow fronds, as a rule, the bands are farther apart, probably because of a more rapid growth of the thallus.

The sterile hairs arise directly from the epidermal cells a short distance back of the distal margin, and soon become about 1 mm. long. Each hair consists of an upright unbranched filament of short cells (fig. 10). In contrast to the rhizoids, growth is entirely basal. There are generally about four bands of hairs present, and behind them are transverse lines representing the place of attachment of hairs which have disappeared. On some plants the sterile hairs are short and more restricted in distribution, while sometimes there are none at all.

In *Zonaria variegata* Richards (7) states that branched rhizoids occur near the base of the underside of the thallus, and that tufts of unbranched hairs are scattered irregularly over the thallus. He finds no bands of hairs in this species, but states that the concentric lines on the thallus represent zones of growth and result from an overlapping of the superficial cells.

Sori

Zonaria Farlowii includes three kinds of individuals: male, female, and asexual. As in *Dictyota dichotoma*, these are alike in size, form, and habit, and are superficially distinguishable from one another only by the character of their reproductive organs (text figs. 2–4). The only previous account of the occurrence of sexual plants in *Zonaria* is that of Sauvageau (8), who described three kinds of individuals in *Z. flava*. Setchell and Gardner (9), who established *Z. Farlowii* as a new species, described only asexual plants, and in their monograph of Pacific Coast algae (10), overlooking Sauvageau's work, state that antheridia and oögonia are not known in *Zonaria*.

The asexual plants of *Zonaria Farlowii*, at all times of the year, greatly exceed the sexual ones in abundance. Often a great deal of searching is necessary in order to find sexual individuals, and in places where only a relatively few plants occur, they are nearly always asexual. Sauvageau (8) found the same situation in *Z. flava* at Teneriffe. Williams (16) reported sexual plants of *Taonia* and *Padina* very rare in the British Isles. Others have found asexual plants of *Dictyota* abundant in certain localities, but few or no sexual ones. This condition seems to be true, in southern California, both of *Dictyota* and of *Neurocarpus*.

In all of the Dictyotaceae the reproductive organs arise from the surface of the thallus. In *Dictyota* only the sexual organs occur in sori, the sporangia being isolated and scattered over the surface of the frond, but in *Zonaria* all three kinds of reproductive organs are borne in sori. The sori arise on both sides of the frond and in the regions between the transverse bands of sterile hairs. On the basis of the character of the sori, the three kinds of individuals are easily recognized. As a rule, the oögonial sori are less extensive than the asexual ones, while the antheridial are the largest of all (text figs. 2–4). The asexual sori are more or less circular in outline, while the sexual sori are generally somewhat irregular, often having the form of a continuous or interrupted zigzag.

Although all three kinds of sori commonly arise on both sides of the frond, usually they are more numerous on one side than the other. Sometimes two sori occur directly opposite each other, but ordinarily this is not the case. On both the sexual and asexual plants, the youngest sori are found approximately 5 mm. behind the anterior margin of the frond, nearly always between the first and second transverse bands of hairs.

The male sori present the appearance of numerous white blisters on the surface of the frond. They are not so conspicuous on living plants as on those which have been fixed. The antheridia are only slightly raised above the level of the thallus. The female sori are brown, and are characterized by an absence of paraphyses.[1] In the asexual sori paraphyses are always

[1] In their description of *Zonaria*, Setchell and Gardner (10, p. 659) say: "Antheridia and aplanospores unknown; oögonia on one or both sides of the thallus, borne in small sori, among paraphyses." This is obviously a typographical error in which the words *aplanospores* and *oögonia* have been transposed, as on the next page the aplanospores of *Z. Farlowii* are correctly designated and described, but the antheridia and oögonia are not mentioned.

present, giving them a speckled appearance, the sporangia being brown and the paraphyses whitish. As a general rule, on the sexual plants the antheridia and oögonia, as the case may be, are all in the same stage of development at a given time, although in two different collections both young and mature antheridia were present on the same plants, while in one of these collections oögonia of different ages occurred together. On the asexual plants, however, young sporangia are found near the distal margin, and successively older sporangia in the sori behind them. Moreover, the peripheral sporangia in a sorus are slightly younger than those in the center, indicating that the sori increase in area after their appearance. All three kinds of sori are covered with a distinct cuticular indusium which breaks when the reproductive organs are ready to shed their contents.

For reasons already stated, it has not yet been possible for the writer to carry on an extensive study at the sea-shore of the relation of external conditions to growth of the thallus and to the production and liberation of reproductive cells. Some data have been secured, however, which indicate an absence of seasonal periodicity, but reveal a definite relation between spore production and the condition of the tides during the various phases of the moon. Asexual plants collected two or three days after the first quarter, and during the spring tides accompanying the full moon, show young sporangia arising immediately behind the distal margin and successively older sporangia farther back. At the time of the last quarter, and during the periods just preceding and following the new moon, practically no young sporangia were found, but only sporangia in more advanced stages on older parts of the fronds. Regarding periodicity in the formation of antheridia and oögonia, not enough data have been obtained to warrant a statement being made at the present time.

Williams (13, 15, 16) found that, in the British Isles, the sporangia of *Dictyota dichotoma* are produced during the summer and all stages occur on the same plant, but the sexual organs show periodicity, the production and liberation of each crop occupying two weeks, the interval between two spring tides. Periodicity in the sexual plants was also studied by Hoyt (4) at North Carolina and by Lewis (5) at Naples. The time relations of this species were found to be very different in the three localities. Williams (16) discovered that the asexual plants of the other British Dictyotaceae, *viz.*, *Taonia*, *Padina*, and *Haliseris* (*Neurocarpus*), also show no periodicity.

Antheridia

The antheridia arise in large groups from the superficial cells of the frond a short distance back of the distal margin, pushing up the cuticle as they develop (Pl. XX, fig. 12). Up to the time of maturity, they are slender, colorless, club-shaped structures with thin cell walls and surrounded by abundant mucilage. No paraphyses are present. As seen in a transverse section of the thallus, one or two antheridia stand directly above each

vertical row of vegetative cells, while in longitudinal section, generally six or eight antheridia are seen above each row. Although all of the antheridia in a sorus are in nearly the same stage of development at a given time, the youngest are always at the periphery. Thus the sorus may increase somewhat in area after its appearance immediately behind the distal margin of the frond.

In the development of an antheridium, the superficial initial divides to form a basal stalk cell and an upper primary spermatogenous cell. Then, by a series of transverse divisions, which seem to occur in centrifugal succession, the latter gives rise to a vertical row of four or five superimposed cells (fig. 12). Additional transverse divisions now occur, and at the same time, vertical walls at right angles to each other make their appearance. As a result, the antheridium consists of approximately ten tiers of cells with four cells in each tier (fig. 13).

Each spermatogenous cell, except a few at the apex of the antheridium, by divisions in either two or three planes now gives rise to four or eight sperm mother cells, respectively. A longitudinal section of an antheridium at this stage of development shows 20–40 tiers of cells (fig. 14), while a cross-section reveals the fact that in some antheridia there are eight cells in each tier, in others 16 (figs. 16, 17). Since each sperm mother cell gives rise to a single sperm, their output varies from about 150 to 650 per antheridium. In *Dictyota*, Williams (15) found the average output of sperms to be about 1,500.

The walls between the sperm mother cells are extremely thin and difficult to demonstrate, but with proper counterstaining may be rendered clearly visible (figs. 15–17). The sperm protoplasts now round up within the cell cavity, and the intervening cell walls, as well as the outer walls of the antheridium, break down, leaving the sperms free in a mass of mucilage (fig. 18). This feature was also observed by Sauvageau (8) in *Zonaria flava*. Very careful observation failed to reveal the presence of cilia on the sperms at this stage. At least one cilium is doubtless present, but is probably closely wrapped about the cell membrane. The mucilage formed by the dissolution of the walls of the sperm mother cells swells, rupturing the indusium and liberating the sperms into the water.

The stalk cells of the developing antheridia are not so dense in contents as the spermatogenous cells, but are much denser than the underlying vegetative cells (figs. 12–14). They are rich in chromatophores and reserve food. After the dehiscence of the antheridia, the stalk cells remain unmodified and regenerate the epidermis beneath the sorus (fig. 18). Each antheridial sorus of *Dictyota dichotoma* has a well defined border of three or more rows of sterile cells, as is well known. In *Zonaria Farlowii* no trace of a similar condition was seen, but according to Sauvageau (8), a limiting layer is sometimes present in *Z. flava*, while in *Z. lobata* the peripheral cells form a sterile collar.

The discovery of motile sperms in the Dictyotaceae was made by Williams (12), who first observed them in *Dictyota* and *Taonia*, and later (16) in *Haliseris* (*Neurocarpus*). When active, the sperms of *Dictyota* were seen to be pyriform and to have a single lateral cilium. Although the actual escape of the sperms was not observed by the writer, the presence of ruptured sori and of many minute swimming cells in filtered sea water containing male plants collected several hours previously, strongly indicates that the sperms of *Zonaria* are motile. Unfortunately the swimming sperms, when seen, were not sufficiently abundant to make it possible to isolate and stain them, and so to determine the number of cilia present.

Oögonia

Like the antheridia, the oögonia arise in groups from the superficial cells of the frond just behind the distal margin. In the male sori, all of the epidermal cells become antheridia, but in the female sori this would not be possible because of the subsequent enlargement of the oögonia. Therefore some of the initials develop into oögonia, while some do not develop at all (fig. 19). Paraphyses are never formed. The development of the oögonia carries up the cuticle, forming an indusium as in the male sori.

The oögonium initials increase in length and slightly in diameter as development proceeds. Their contents become dense, and rich in chromatophores and reserve food. At the same time the nucleus of each increases to about twice its former diameter. At this stage polar radiations, such as occur in the apical cells, are visible with proper staining (fig. 19). After the oögonia have undergone considerable elongation, a small stalk cell is cut off from each, the rest of the oögonium constituting a single large egg. The diameter of the egg nucleus is nearly 20 μ, or approximately four times greater than that of the oögonium initial. The cytoplasm is now somewhat vacuolate, and the chromatophores still numerous (fig. 20).

Following each collection, a few male and female plants were placed in a large jar of sea water. The latter were examined at frequent intervals with a high-powered dissecting microscope, but at no time were the eggs seen to be escaping. On several occasions the plants were kept in the jars for as long as a month, and while they remained alive during this period, there was little or no growth of the thallus and no escape of the eggs. Obviously some condition necessary for the liberation of the gametes, present in the ocean, was absent in the cultures.

Sauvageau (8) states that in *Zonaria flava* the oögonia have no stalk cell. He observed the escape of the large non-motile egg, but did not see fertilization. Where female plants were isolated in cultures, Sauvageau noticed some cases of parthenogenesis after the escape of the eggs, but never before. In *Padina variegata*, Wolfe (17) found that "unfertilized eggs divide freely, producing a cell body of varying size, but which invariably fails to mature." Williams (15) demonstrated that fertilization of the

non-motile eggs of *Dictyota dichotoma* takes place after their escape from the oögonia, the sperms being attracted to them. Germination proceeds at once, but if fertilization does not occur, the eggs develop parthenogenetically. Only a few divisions take place, however, and then the eggs die. Williams later (16) found this true also of *Haliseris* (*Neurocarpus*).

Sporangia

The sporangia, like the sexual organs, are epidermal and subcuticular in origin and centrifugal in development within the sori. A notable difference is the presence of numerous paraphyses among the sporangia. In the development of a sporangium, one of the superficial cells of the frond enlarges, pushing up the cuticle (Pl. XXI, figs. 21, 22). Its nucleus increases in diameter two or three times, and its cell wall remains thin. After the length of the sporangium initial has become two or three times its diameter, the nucleus divides, but no basal cell is cut off as happens in the development of the antheridium and oögonium (fig. 23). Instead, with a further increase in size, a second mitosis occurs, resulting in four free nuclei (fig. 24). The sporangium now undergoes a marked enlargement, becoming pyriform, and each of its nuclei undergoes another division to form eight (fig. 25).

When the eight nuclei are first formed they are in no way peculiar, but as the sporangium becomes larger, some, and later all, of them become irregular in outline and increasingly more dense, finally staining very deeply. This condition, illustrated by figure 26, is very characteristic and seems always to occur, but is ephemeral, as the nuclei soon return to their original condition. Perhaps, at this stage of development, the nuclei are so affected by the fixing reagents employed that they assume an abnormal appearance.

Delicate walls now appear in the sporangium, cutting off eight spores. Figure 27 shows the arrangement of the spores as seen in a median longitudinal section. By this time the paraphyses, by a series of parallel divisions, have reached a length slightly in excess of the sporangia. They are clavate in form, each one consisting of a filament of five to seven cells. They contain many chromatophores and much reserve food material. At this stage the cuticular indusium has become broken and the sporangia are ready to shed their spores.

After each collecting trip some of the asexual plants, kept in jars of sea water, were examined with a dissecting microscope, and while often many empty sporangia were present, the escape of the spores was not seen. Zoöspores have never been reported in the Dictyotaceae, and so it is practically certain that the spores of *Zonaria Farlowii* are aplanospores. Sauvageau (8) describes the spores of *Z. flava* as naked and non-motile.

Although, as a rule, the spores are shed soon after being formed, as evidenced by the presence of numerous empty sporangia surrounded by

others in the stage of development shown by figure 27, in some cases they germinate *in situ*, a feature also observed by Sauvageau. Each spore forms a wall about itself, separates slightly from its neighbors, and by irregular internal segmentation gives rise to a small group of delicate-walled cells (Pl. XXII, fig. 31). Such stages were seen only on material from a few collections, and on older parts of the plants, and so probably are not of common occurrence. When grown in culture, the asexual plants lived for several months, but did not increase in length or shed their spores. Thus it was not possible to study spore germination. According to Sauvageau (8), the spores of *Zonaria flava* germinate quickly, first producing a rhizoid, and then a foliaceous, flabellate thallus one layer of cells in thickness.

In *Dictyota* and most of the other genera of Dictyotaceae, the sporangium mother cell cuts off a stalk cell and forms four spores. Because of the formation of "tetraspores," an affinity with the Rhodophyceae has been claimed for the Dictyotaceae by many authors. Sauvageau, who studied three species of *Zonaria*, found differences among them with respect to spore formation. *Z. flava* produces eight spores, a feature first observed by Bornet (1), who compared them to the eight eggs of *Fucus*. The sporangia of *Z. lobata* form four spores, but in *Z. variegata*, a very widespread species, a peculiar condition exists. From December 24 to January 9 Sauvageau collected, at Teneriffe, mature erect plants with sporangia containing four spores arranged as in *Dictyota*. Shortly thereafter he collected creeping plants with sporangia containing eight spores, the erect plants being at that time sterile. A month later he found both kinds of sporangia on the same thallus, but by the next day the four spores had divided to form eight. Later he found only sporangia with eight spores. Since the erect plants of *Z. variegata* produced tetrasporangia in December and January, and octosporangia in February, Sauvageau concluded that the latter represent the typical form of asexual reproduction, as in *Z. flava*, and the former the accidental form.

Reduction of Chromosomes

Accompanying the enlargement of the sporangium initial, which in *Zonaria* is also the spore mother cell, numerous short astral radiations appear at the distal pole of its nucleus (Pl. XXI, figs. 21, 22). In some cases a minute centrosome seemed to be present, but usually none could be seen. The radiations are extremely delicate and are rendered visible only after careful counterstaining of the sections. In *Dictyota dichotoma* the diameter of the spore mother cell nucleus is about twice that of *Zonaria Farlowii*. In the former species, according to Mottier (6), no asters or centrosomes are present in the resting nuclei, but as the spore mother cell increases in size "there appear on opposite sides of the nucleus two large, sharply defined clusters or asters of kinoplasmic fibers radiating from a rod-shaped . . . centrosome." Williams (14), working on the same form,

found a centrosome at each pole of the nucleus of the sporangium initial, but saw radiations only at the distal pole.

During the course of the present investigation, a number of cases of mitosis were seen in the first division of the spore mother cell nucleus. No prophases were found, but many metaphases and several anaphases were critically examined. The first division of the spore mother cell is accompanied by the heterotypic mitosis (fig. 28). An equatorial view of the metaphase shows approximately 12 bivalent chromosomes arranged in a circle. At this stage the chromosomes are short and thick, and while their extremely small size renders them difficult to study, often their paired character is very evident. Where the entire figure lies in one section, counting is difficult, but where the chromosomes are distributed about equally between two consecutive sections, there is little or no overlapping and the total number can readily be estimated. At the anaphase 12 univalent chromosomes pass to each pole (fig. 29).

As in *Dictyota* and *Padina*, a bipolar, intranuclear spindle is organized whose long axis lies at right angles to the surface of the thallus. An aster composed of numerous delicate fibers, and probably also a centrosome, are present at each pole (figs. 28, 29). Polar radiations are present during all of the free-nuclear stages in sporogenesis, but seem to disappear when walls are formed. In *Dictyota*, Mottier (6) found that, after the spindle is formed, the polar radiations become inconspicuous and the centrosomes smaller, but that centrosomes are unquestionably present during all stages of the two divisions of the spore mother cell, and also in the three or four divisions involved in the germination of the tetraspore.

Heretofore chromosome numbers in the Dictyotaceae have been determined only for *Dictyota* and *Padina*. In the former, Mottier (6) counted 16 bivalent chromosomes at the first mitosis of the spore mother cell, and noted that each was in the form of a ring or a short thick U. In the vegetative cells of the asexual plants, he found the chromosomes "short, bent, slightly U-shaped, and about 32 in number." Williams (14), adding many details to Mottier's account, found 16 bivalent chromosomes at the first division of the spore mother cell both in *Dictyota* and *Padina*. He found the reduced number in the second division of the spore mother cell of *Dictyota* and also in the germinating tetraspore. Williams also (15) studied the stalk-cell division of the oögonium initial, finding 16 U-shaped chromosomes. He counted the same number in the antheridium initial and 32 in the germinating zygote. Georgevitch (3), studying *Padina Pavonia*, counted 24 chromosomes at the metaphase of the first division of the spore mother cell, and found 12 going to each pole. Carter's (2) investigation of this species, however, definitely confirmed Williams' previous count of 16 and 32.

In the present investigation no instance was seen of the second (homotypic) mitosis in the spore mother cell, but a number of sporangia were

found with four nuclei in metaphase. Both polar and equatorial views were presented. Apparently 12 unpaired chromosomes, the reduced number, are present at this division (fig. 30). They are long and slender, and assume a very different appearance from that of the chromosomes formed during the heterotypic mitosis.

Adventitious Thalli

On all three kinds of plants there arise, among the rhizoids along the median line of the thallus, outgrowths which develop into miniature plants (text fig. 1; Pl. XXII, fig. 32). They appear approximately 3 cm. behind the apical margin and are formed in large numbers on both sides of the thallus. These plantlets reach a length of about 2 cm. and develop a small group of rhizoids at the base. After reaching this size they may easily be pulled off the main thallus, and so presumably become detached to form independent plants. Before doing so, however, those on the asexual plants may produce a few sporangial sori of their own, while those on the male and female plants may produce, respectively, antheridial and oögonial sori. Plantlets were found on practically all of the material collected for the present investigation.

In the development of an adventitious thallus, an epidermal cell enlarges without pushing up the cuticle (fig. 33). Transverse divisions then occur, followed by vertical divisions, and soon a small thallus is formed (figs. 34–37). The youngest stages have cytoplasm which does not stain deeply, and relatively few chromatophores are present. The terminal cell of the older stages has a larger nucleus than the others, and probably alone is meristematic.

Sauvageau (8) found that, in *Zonaria flava*, the foliaceous parts of the thallus disappear on old plants, and that new fronds arise from the caulescent stumps. These probably correspond to the adventitious thalli described above.

Summary

1. *Zonaria Farlowii* is a southern California species locally abundant in the vicinity of Laguna Beach. It grows in tidepools attached to rocks.

2. The plants, reaching a length of 15 cm., are erect, fan-like, and profusely branched. They are mostly frond-like, but stipe-like below with a disk-shaped basal holdfast composed of a mass of rhizoids.

3. The fronds are thin, flat, and flabellately divided, the stipes costate by the abundance of branched rhizoids. Transverse bands of erect unbranched hairs occur on the fronds.

4. Growth occurs at the rounded distal margin through the activity of a row of initials. Branching results from local death of marginal cells.

5. The marginal initials cut off posterior segments in one plane, from which, by successive divisions parallel to the surface, the fronds become eight layers of cells thick. The superficial cells further divide at right angles to the surface, but the central cells do not.

6. Three kinds of individuals, superficially alike, are present, *viz.*, male, female, and asexual. The sexual plants are comparatively rare.

7. Antheridia, oögonia, and sporangia are borne in sori occurring on both sides of the fronds between the transverse bands of hairs. They arise from superficial cells a short distance behind the apical margin. The sori increase slightly in area after their appearance.

8. Paraphyses occur among the sporangia, but not among the sexual organs.

9. Successive stages in the development of the reproductive organs are found on the asexual plants, but ordinarily not on the sexual ones. Young sporangia arise only just after the period of the lunar month beginning two or three days after the first quarter and ending soon after the full moon.

10. All three kinds of reproductive organs are subcuticular in origin, the cuticle forming an indusium which is ruptured when they are ripe.

11. In both the antheridium and oögonium, the first wall is transverse, cutting off a stalk cell. The primary oögenous cell, by a considerable increase in size, directly becomes the egg. The primary spermatogenous cell, by transverse and vertical divisions, gives rise to 20–40 tiers with 8 or 16 small cells in each.

12. The sperms, each derived from a single mother cell, lie free in a mass of mucilage formed by the breaking down of the antheridial walls. The output of sperms, per antheridium, is approximately 150–650.

13. No stalk cell is formed in the sporangium. The initial, which therefore is also the spore mother cell, becomes large and undergoes three successive free-nuclear divisions, the first of which is the heterotypic mitosis.

14. The eight nuclei become oriented in a characteristic manner, and walls then appear to form eight large aplanospores. These are usually soon shed, but may germinate *in situ*.

15. Astral radiations, and probably also centrosomes, appear in connection with the division of the apical cells, the oögonium initials, and the three free-nuclear divisions of the spore mother cell.

16. The chromosome numbers are 12 and 24. Counts were made at the first and third mitoses in the spore mother cell.

17. Adventitious fronds appear along the median line of the thallus, become detached, and form independent plants. They arise on all three kinds of individuals.

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EXPLANATION OF PLATES

PLATE XIX

FIG. 5. Median longitudinal section of thallus. × 150.

FIG. 6. Portion of apical cell of same, showing astral radiations at distal pole of nucleus. × 500.

FIG. 7. Section cut parallel to surface of thallus. × 150.

FIG. 8. Portion of epidermis cut parallel to surface of thallus. × 375.

FIG. 9. Transverse section of thallus. × 375.

FIG. 10. Transverse section of portion of thallus, showing origin of sterile hairs. × 375.

FIG. 11. A few rhizoids. × 75.

PLATE XX

FIG. 12. Transverse section of portion of young male sorus, showing early stages in development of antheridia. × 375.

FIG. 13. The same, later stage. × 375.

FIG. 14. The same, showing formation of sperm mother cells. × 375.

FIG. 15. Longitudinal section of portion of antheridium with sperm mother cells. × 1000.

FIGS. 16, 17. Transverse sections of same. × 1000.

FIG. 18. Transverse section of portion of thallus, showing ripe sperms free in mucilaginous matrix. × 375.

FIG. 19. Transverse section of portion of young female sorus, showing young oögonia before cutting off of stalk cell. × 375.

FIG. 20. Transverse section of portion of mature female sorus, showing ripe oögonia. × 375.

PLATE XXI

FIGS. 21, 22. Transverse section of portion of sporangial sorus, showing sporangium initial and paraphyses. ×375.

FIG. 23. First division of spore mother cell nucleus. × 375.

FIG. 24. Second division of same. × 375.

FIG. 25. Third division of same. Nuclei in four sections. × 375.

FIG. 26. Spore mother cell with nuclei in characteristic but peculiar condition. Two more nuclei present in other sections of series, those shown occurring in three sections. × 375.

FIG. 27. Mature sporangium showing wall formation. Another nucleus occurs above and still another below the plane of those shown here. × 375.

FIG. 28. Metaphase of heterotypic mitosis in spore mother cell. The next section shows five bivalent chromosomes. × 1,500.

FIG. 29. Anaphase of heterotypic mitosis. × 1,500.

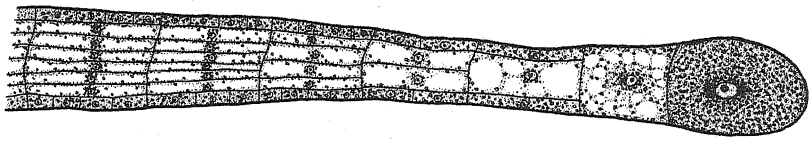
FIG. 30. Polar view of chromosomes at metaphase of third mitosis in spore mother cell. × 1,500.

PLATE XXII

FIG. 31. Germination of aplanospores before being shed, an unusual condition. × 375.

FIG. 32. Older portion of frond, showing adventitious plantlets arising along median line. × 2.

FIGS. 33–37. Stages in the development of adventitious frond. × 375.



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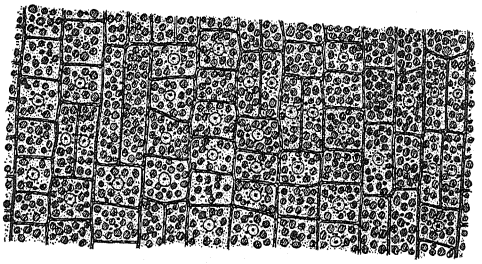

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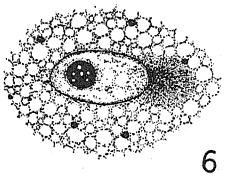

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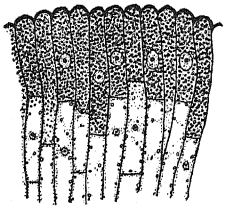

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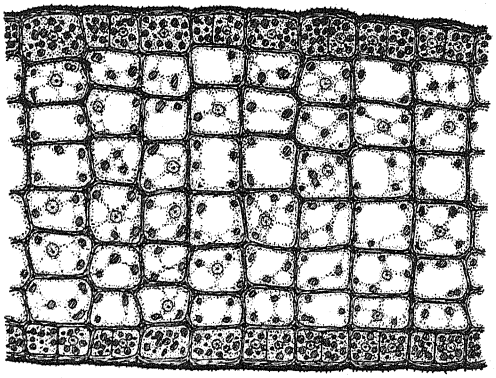

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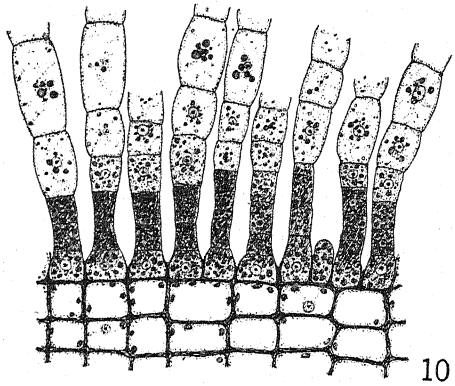

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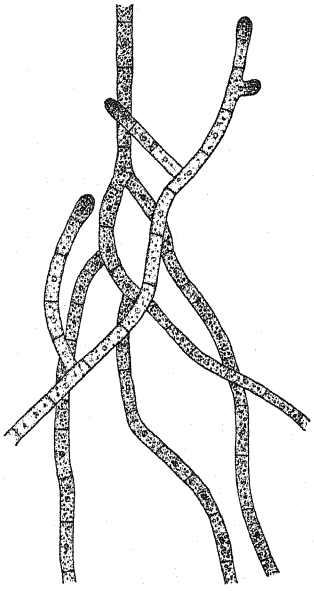

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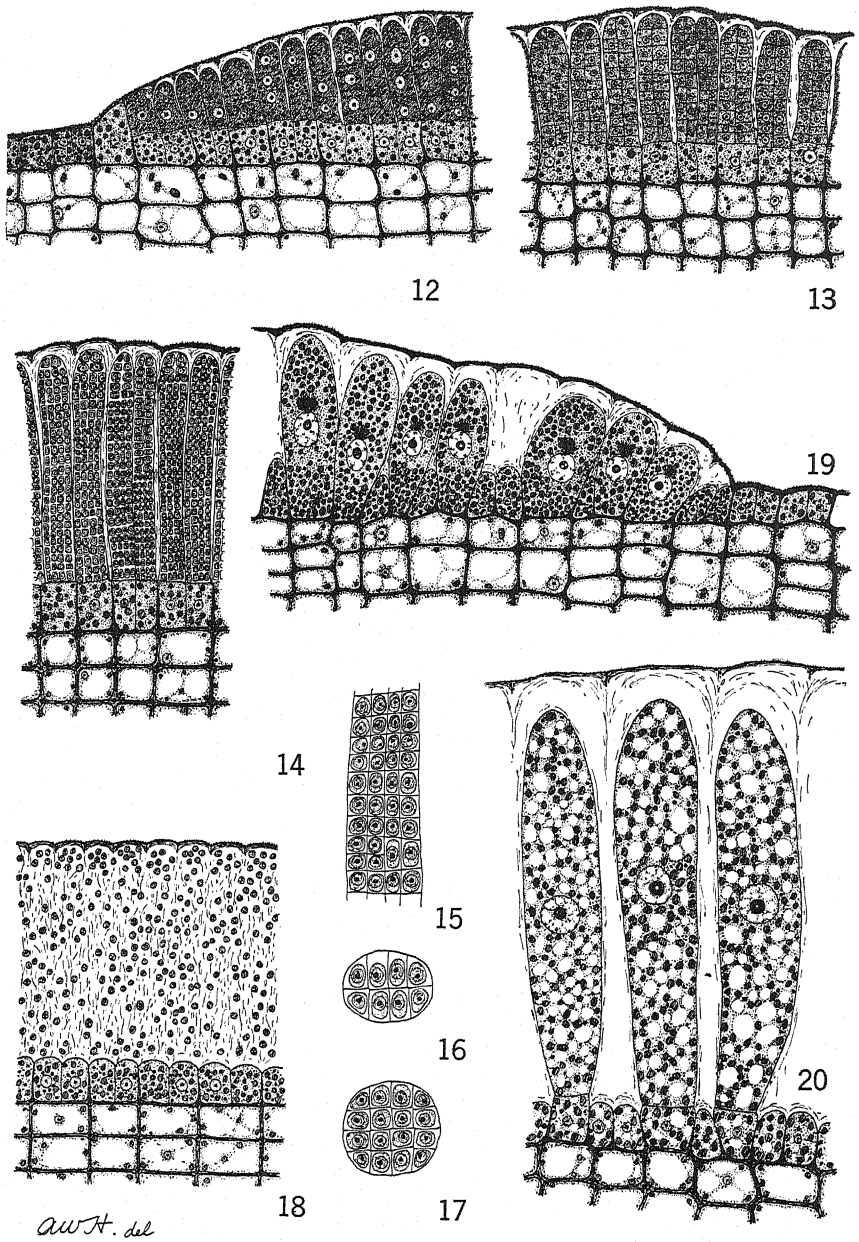

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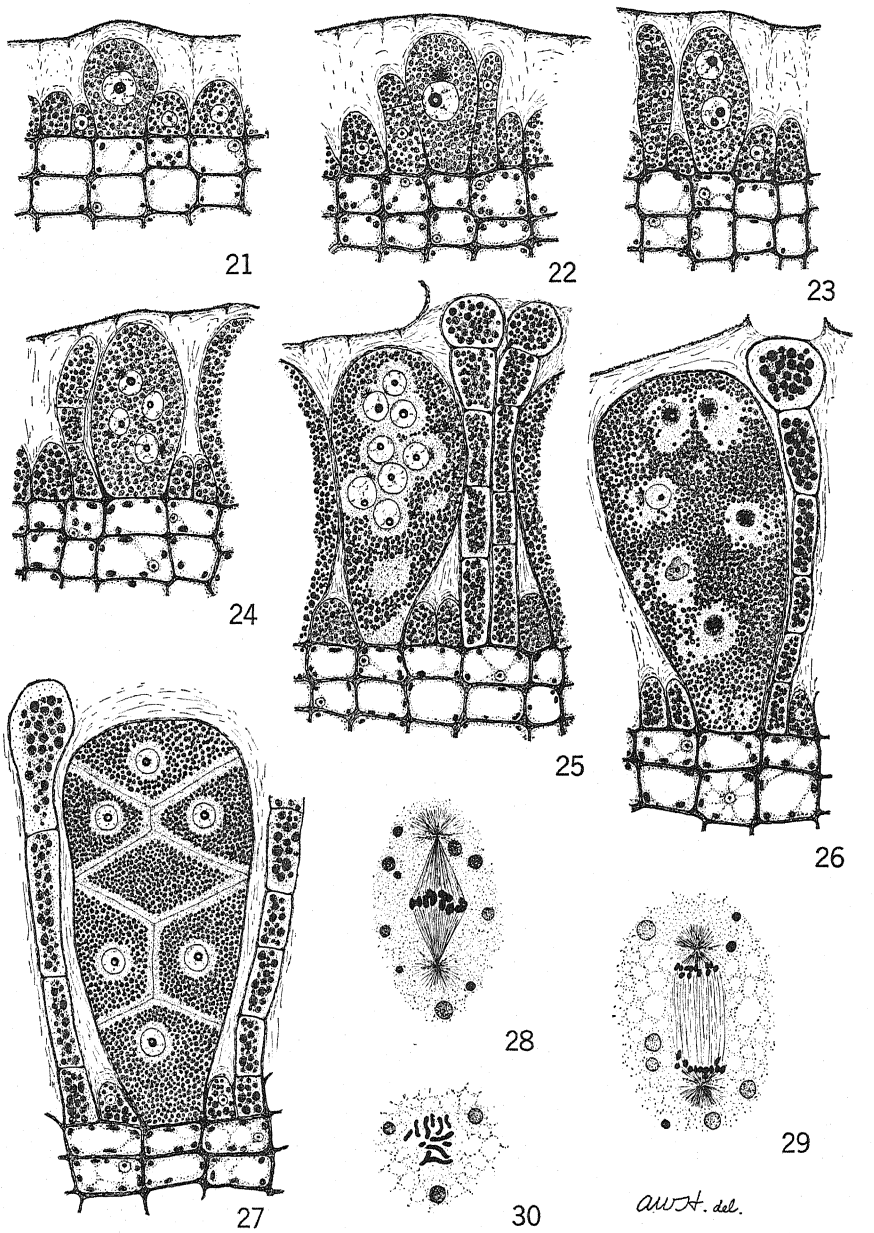

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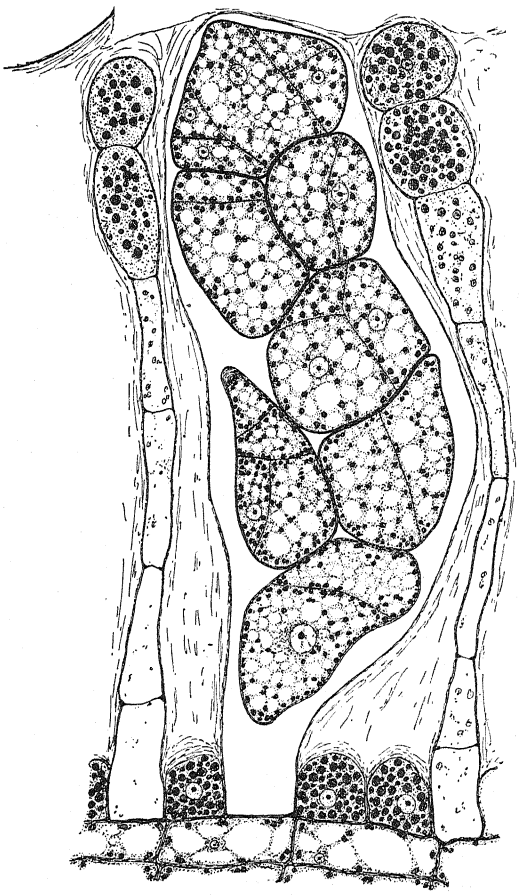

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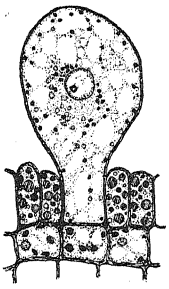

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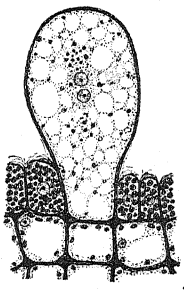

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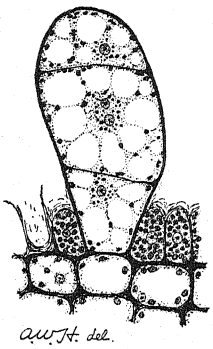

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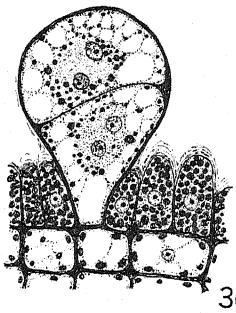

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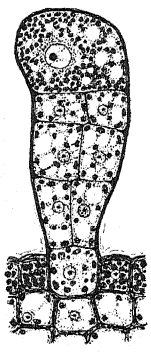

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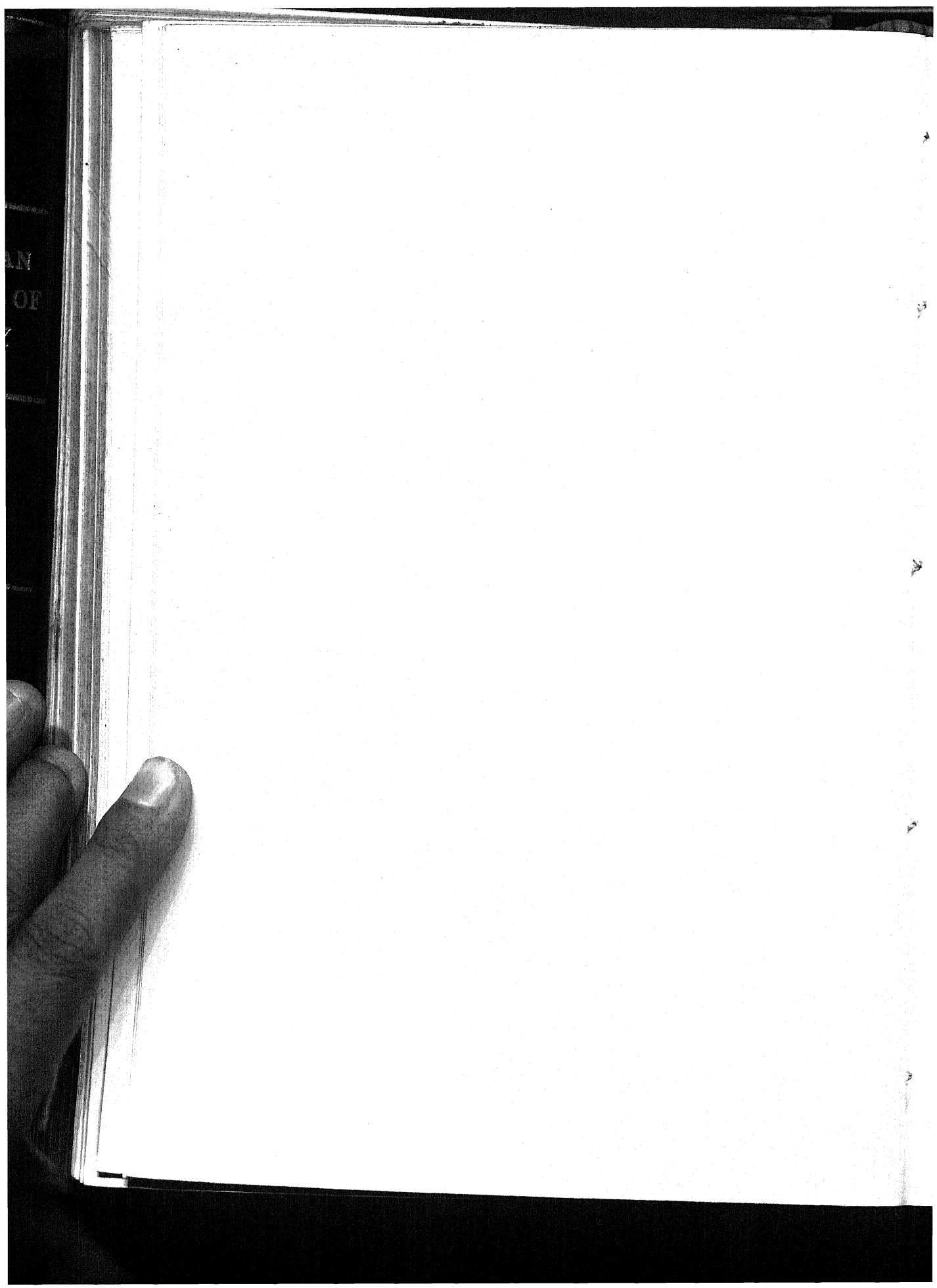

THE INTERRELATION BETWEEN SUBSTANCES IN PLANTS: ESSENTIAL OILS AND RESINS, CYANOGEN AND OXALATE

JAMES B. MCNAIR

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The objects of this paper are (1) to show the probability of resin formation in plants from essential oils and (2) to demonstrate the possibility of oxalate production from cyanogen.

ESSENTIAL OILS AND RESINS

If resins are formed from essential oils then: (1) All resin-bearing plants must contain essential oils, and (2) substances must be present in essential oils that will interact with themselves or other substances to form resins.

Definitions of Resin and Essential Oil

Natural resins are in general brittle exudations of plant tissues, formed either normally or as a result of pathogenic conditions, or sometimes artificially induced. Their chemical constitution is generally highly complex, with the exact constituents still uncertain, but their ultimate analysis shows carbon and hydrogen with a small amount of oxygen. They are characterized by chemical stability and inertness, not being readily attacked by acids or alkalies. They are insoluble in water and inorganic liquids, but are more or less soluble in numerous organic solvents. They have an amorphous structure (rarely crystalline), become sticky when heated, often at comparatively low temperatures, and are fusible with no sharp melting points.

Volatile, essential, or ethereal oils are aromatic volatile substances of an oily nature usually obtained by the distillation of vegetable products with steam. They are generally liquid, though sometimes semi-solid at ordinary temperatures, slightly soluble in water, soluble in alcohol, ether, benzene, light petroleum, and most organic solvents. They are found in all parts of the plant or tree, some occurring in the woody stems or roots, others in the bark, leaves, flowers, and fruits. Many essential oils are complex mixtures, containing constituents belonging to various classes of organic compounds, as hydrocarbons, alcohols including phenols, aldehydes, acids, esters, phenol ethers, ketones, lactones, quinones, oxides, bases, sulphides, mercaptans, nitriles, and isothiocyanates.

Distribution of Essential Oils in Plant Families

The essential oil-bearing families of the gymnosperms and angiosperms as recently listed by Gildemeister (1922) and Wolff (1927) may be climatically divided according to Engler and Gilg and Willis. By this classification they

are, tropical: Pandanaceae, Palmae, Araceae, Zingiberaceae, Urticaceae, Menispermaceae, Anonaceae, Myristicaceae, Monimiaceae, Lauraceae, Pittosporaceae, Erythroxylaceae, Humiriaceae, Zygophyllaceae, Burseraceae, Meliaceae, Anacardiaceae, Sapindaceae, Gonystilaceae, Tiliaceae, Sterculiaceae, Guttiferae, Dipterocarpaceae, Winteraceae, Myrtaceae, Apocynaceae, Asclepiadaceae, Convolvulaceae, Acanthaceae, Rubiaceae, Piperaceae, Turneraceae, Caricaceae, Vitaceae, Orchidaceae, Moraceae, Araliaceae, and Verbenaceae; tropical-subtropical: Tropaeolaceae, Theaceae, Geraniaceae, Iridaceae, and Magnoliaceae; subtropical: Myricaceae and Hamamelidaceae; subtropical-temperate: Taxaceae; temperate: Resedaceae, Caprifoliaceae, Salicaceae, Juglandaceae, Betulaceae, Caryophyllaceae, Ranunculaceae, Papaveraceae, Cruciferae, Saxifragaceae, Cistaceae, Umbelliferae, Primulaceae, Gentianaceae, Pinaceae, Polygonaceae, Pirolaceae; and widely distributed: Santalaceae, Aristolochiaceae, Rutaceae, Euphorbiaceae, Lythraceae, Solanaceae, Malvaceae, Liliaceae, Rosaceae, Ericaceae, Labiatae, Valerianaceae, Gramineae, Ulmaceae, Chenopodiaceae, Leguminosae, Polygalaceae, Aquifoliaceae, Celastraceae, Violaceae, Thymelaeaceae, Oleaceae, Scrophulariaceae, and Compositae.

According to the foregoing list essential oils are found in 87, or 29.49 percent, of the 295 plant families listed by Engler and Gilg. Of the families containing essential oils, 38, or 43.6 percent, are mainly tropical in habitat; 5, or 5.74 percent, are tropical-subtropical; 2, or 2.29 percent, are subtropical; 1, or 1.14 percent, is subtropical-temperate; 17, or 19.51 percent, are temperate; and 24, or 27.58 percent, are widely distributed in life zones.

Of a total of 153 tropical families 38, or 24.84 percent, have essential oils; of 15 tropical-subtropical families 5, or 33.33 percent, have essential oils; of 8 subtropical families 2, or 25.0 percent, have essential oils; of 7 subtropical-temperate families 1, or 14.28 percent, has essential oils; of 50 temperate families 17, or 34.00 percent, have essential oils, and of 57 families of widely distributed habitats 24, or 42.10 percent, have essential oils.

Distribution of Resins in Plant Families

The plant families that contain appreciable amounts of resins are, according to Wolff (Wiesner, 1927), tropical: Palmae, Myrothamnaceae, Zygophyllaceae, Burseraceae, Anacardiaceae, Sapindaceae, Guttiferae, Dipterocarpaceae, Myrtaceae, Convolvulaceae, Rubiaceae, Moraceae, and Araliaceae; subtropical: Hamamelidaceae; temperate: Caprifoliaceae, Juglandaceae, Betulaceae, Cistaceae, Umbelliferae, and Pinaceae; and widely distributed: Euphorbiaceae, Styracaceae, Liliaceae, Leguminosae, Rhamnaceae, and Compositae.

It is apparent from the list just completed that 26 families are known to produce appreciable amounts of resin. Of these families 13, or 50.0 percent, are tropical; 1, or 3.8 percent, is subtropical; 6, or 23.0 percent, are temperate; and 6, or 23.0 percent, are widely distributed in habitat.

The climatic distribution of resin families may be compared with the climatic distribution of the total numbers of plant families. Making use of the climatic distribution of these as determined from Engler and Gilg and Willis it becomes apparent that of the 295 families of gymnosperms and angiosperms 26, or 8.81 percent, contain resin. Of a total number of 153 tropical families 13, or 8.43 percent, have resins; of 8 subtropical families 1, or 12.5 percent, has resin; of 50 temperate families 6, or 12.0 percent, have resins; and of 57 families of widely distributed habitats 6, or 10.5 percent, have resins.

Distribution of Essential Oils and Resins Compared

Of the 87 essential oil-containing families only 24, or 27.81 percent, have resin in appreciable quantity. It is therefore evident that it is not necessary for an essential oil-bearing family to have resin in large amount. Of the 27 resin-bearing families 24 have also essential oils according to Gildemeister. The three resin-producing families which apparently are not associated with appreciable amounts of essential oils are the Myrothamnaceae, Styracaceae, and Rhamnaceae. Before definitely deciding that these families do not contain essential oils they should be further investigated.

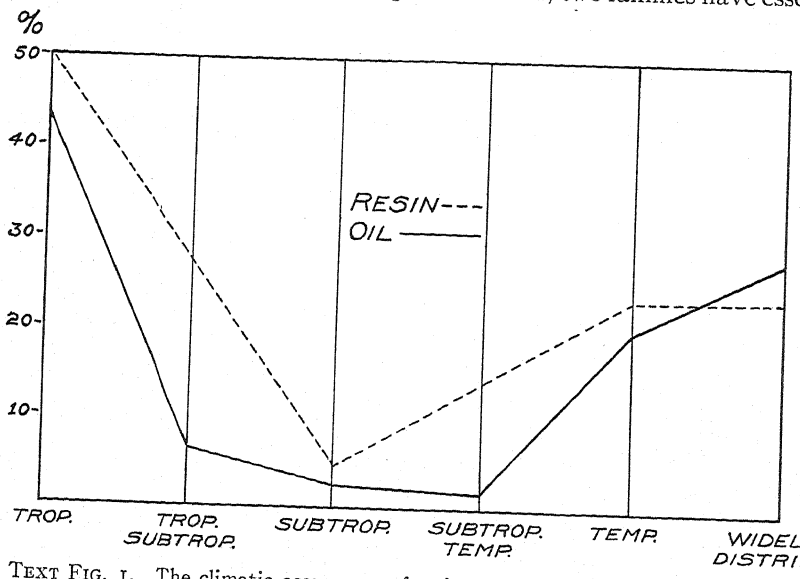
The Myrothamnaceae are closely related to the Hamamelidaceae and Pittosporaceae, both of which contain essential oils. The only plant of the Myrothamnaceae that is accredited with yielding resin is *Myrothamnus flabelliformis* Welw. The resin obtained from this plant is, according to Niedenzu (Engler and Prantl), a balsam. As balsams are by definition mixtures of resins and essential oils it is apparent that an essential oil is present in this plant although not cited by Gildemeister.

Two plants are considered as furnishing the resin of the Styracaceae. These are *Styrax Benzoin* Dryand., which yields gum benzoin, and *Styrax officinale* L., which gives true storax. Gum benzoin consists principally of benzoic acid, partly free and partly combined with resin alcohols, which have been named benzoresinol and siaresinotannol. Vanillin is present in traces and in Sumatra benzoin, about half the free acids consist of cinnamic acid. Small quantities of vanillin, styrol, styracine, phenylpropyl cinnamate, and benzaldehyde are also present in Sumatra benzoin. All of these substances except benzoresinol and siaresinotannol are present in essential oils. Gum benzoin also is defined as a balsamic resin containing essential oil. The other resin known to this family is true storax from *Styrax officinale* L. This substance is similar to benzoin in composition.

The third resin-containing family which according to Gildemeister is not considered as containing essential oil is the Rhamnaceae. The genus to which it owes its inclusion in the list of resin-producing families is *Zizyphus*. This genus produces a water-soluble gum but does not naturally produce any resin (Watt). The substance because of which *Zizyphus* is classed among the resin families is shellac. Shellac is not purely a vegetable product but is

the secretion of the Lac insect, *Tachardia lacca*, which subsists upon the vegetable sap of certain trees. Both hard and soft wooded trees are capable of acting as hosts, some of which yield resin and others do not. Generally, the soft wood non-resinous trees are preferred. The chemical composition of shellac (Barry, Drummond, and Morrell, 1926) is more like that of a wax than a resin as indicated by mono-, di-, and tri-hydroxypalmitic acid contained in the portion soluble in cold alcohol and the myristic esters of melissic, palmitic, and cerotic acids which constitute the portion of shellac insoluble in cold alcohol. The presence of such material would not necessitate shellac formation from an essential oil.

It is evident, therefore, that of the three resin-containing families not listed by Gildemeister as also having essential oils, two families have essential



TEXT FIG. 1. The climatic occurrence of resins and essential oils in plant families.

oils and the third does not produce a true resin. It may therefore be concluded that 100 percent of the resin-producing families produce essential oils.

It is of interest also to notice that although there are three times as many essential oil families as resin families there is a close parallelism between the percentages of essential oil families and resin families in similar habitats. For instance, of the oil-containing families, 44 percent are tropical, of the resin-containing, 50 percent; of oil, 2 percent are subtropical, of resin, 4 percent; of oil, 19 percent are temperate, of resin 23 percent; of oil, 27 percent are widely distributed, of resin, 23 percent.

Mutual Association of Essential Oil and Resin in the Same Plant

It has been shown that essential oils occur in the same families as resins but in order to demonstrate that resin and oil may be mutually associated in

TABLE I. *Some Resins Naturally Incorporated with Essential Oils*

Resin Name	Family	% Oil	Oil Constituents
Mastic	Anacardiaceae	1–3	Pinene
Bisabol Myrrh	Burseraceae	7	Bisabolene
Opopanax	Burseraceae	5–10	Bisabolene (resinifies quickly in air)
Conima	Burseraceae	traces	Coninene
Manila elemi	Burseraceae	20–30	Phellandrene, dipentene, elemicin (a phenol ether)
Ma'ali resin	Burseraceae	16	Ma'ali alcohol (sesquiterpene alcohol)
African elemi	Burseraceae	11	Phellandrene
Dammar	Dipterocarpaceae	1	— — —
Lagan balsam	Dipterocarpaceae	10–22	Caryophllene
Gurjun balsam	Dipterocarpaceae		Gurjunene (hydrocarbon)
Guaiac resin	Zygophyllaceae	0.66	— — —
Amer. Storax	Hamamelidaceae	7	Styrene, vanillin, cinnamyl cinnamate, phenyl propyl cinnamate
Storax	Hamamelidaceae		Phenylethylene (styrene), styrocamphene, ethyl cinnamate, benzyl cinnamate, naphthalene, phenylpropyl cinnamate, cinnamyl cinnamate, vanillin
Honduras balsam	Hamamelidaceae	15–20	Cinnamic acid, cinnamic alcohol, phenyl propyl alcohol, C_8H_8, C_5H_{10}, C_9H_{12}
Sandarac	Pinaceae		Pinene, diterpene
Kauri Copal	Pinaceae		Pinene, dipentene, isoprene
Manila Copal	Pinaceae		Pinene, dipentene, limonene, camphene
Araucaria	Pinaceae	6	— — —
Rosin	Pinaceae		Pinene, dipentene, cymene, camphene
Canada Balsam	Pinaceae		Pinene
Sagapenum	Umbelliferae	19	
Galbanum	Umbelliferae	10–22	Pinene, cadinene
Asafoetida	Umbelliferae		Pinene
Labdanum	Cistaceae	0.91	Acetophenone, trimethyl hexavone, guajol
Yucatan elemi	Burseraceae	8–10	
Copaifera Balsam	Leguminosae	85	Cadinene, caryophyllene
Angola Copal	Leguminosae	2	
Benin Copal	Leguminosae	3	
Copaifera Balsam	Leguminosae	40–72	Cadinene, sesquiterpene alcohol, sesquiterpene
Illurin Balsam	Leguminosae	37–46	Cadinene, caryophyllene
Tolu Balsam	Leguminosae	1–7	Phellandrene, farnesol, benzyl benzoate, benzyl cinnamate
Peru Balsam	Leguminosae		Farnesol, benzyl benzoate, benzyl cinnamate, vanillin, peruviol, dihydrobenzoic acid
Xanthorrhoea	Liliaceae	33–37	Styrene, cinnamic acid free and as ester.

the same plant the data in table I are presented, which lists some of the common resins together with the percentages and chemical constituents of their mechanically incorporated essential oils which have been separated from the resins by steam distillation.

Location of Essential Oil and Resin in the Plant Structure

When the location of essential oils and resins is investigated in plant structures, both substances are found in similar anatomical structures

(DeBary, 1884; Haberlandt, 1914), namely in secretory glands, excretory reservoirs, and parenchyma cells, located in leaves, flowers, stems, and roots. Both compounds are frequently found in the same cellular enclosure.

Resin-forming Substances in Essential Oils

One of the best ways to determine the possibility of resin formation from essential oils is to study the formation of artificial resins. The classification of artificial resins based on the reactions that occur during formation has been used by Scheiber and Sandig (1929) and by Carothers (1929), and is as follows:

(1) Polymerization. Examples: Olefins and poly-olefins, unsaturated hydrocarbons, azo compounds.

(2) Condensation. Examples: Poly-esters, phenol-aldehydes, ketone-aldehydes, hydrocarbon-aldehydes, alcohol-phenols, phenols-sulfur chlorid.

There are many of these chemical groups present in essential oils, *e.g.*, olefins (octylene); olefinic terpenes which show a great tendency to resinify (myrcene, ocimene); alicyclic hydrocarbons (α-pinene, which like many terpenes upon standing partly resinifies); sesquiterpenes (ceylon citronella oil, which is very unstable when exposed to the air and resinifies within a few days, as does cadinene with the formation of a polymerization product); esters (benzyl acetate, cinnamyl acetate, geranyl acetate, linalyl acetate, eugenyl acetate); phenols (anol, chavicol, charvacrol, thymol, anethol, safrol, estragol); aldehydes (citronellal, geranial, santalal, citral), ketones (anisaceton, menthon, fenchon, camphor, pulegone); and alcohols (menthanol, benzyl-alcohol, citronellol, geraniol, linalool, menthol, terpinol).

It seems likely that natural resins may therefore be formed from constituents of essential oils. For example, there is the oil in the fruit of *Pittosporum resiniferum* Hemsl., which contains heptane and dihydroterpene and rapidly resinifies in the air. The essential oil exudate from the trunk of *Nectandra Caparrapi* Pharm. ex Wehmer contains the sesquiterpene alcohol caparrapiol and the hydrocarbon caparrapene which readily polymerize with heat to form a resin. There is also tolu balsam from *Myroxylon toluiferum* H.B.K. which hardens in time and contains the sesquiterpene phellandrene, the alcohol farnesol, and benzyl esters of benzoic and cinnamic acids.

Function of Essential Oils and Resin

Essential oils apparently serve as solvents for, transporting agents for, and mother substances of, resins. As nearly twice as many essential oil and resin families occur in the tropics as in the temperate zone, these two substances, in accordance with the abundance of their distribution, are apparently useful in both climates, but more so in the tropics.

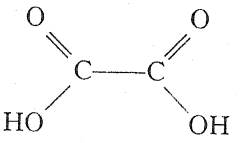
Cyanogen and Oxalate

The simultaneous occurrence of prussic acid, protein matter, and calcium oxalate in cells in *Pangium edule* has been noticed by Treub (1907, 1910).

The finding of two nitrogenous compounds in a cell is not remarkable but the discovery of prussic acid in the presence of an oxalate leads to conjectures regarding the chemical origin of the oxalate.

Chemical Basis

From a chemical standpoint cyanogen is closely related to oxalic acid, for if ammonium oxalate be heated with a dehydrator such as phosphoric oxid, cyanogen is produced. Inversely, if cyanogen is dissolved in hydrochloric acid, ammonium oxalate is formed by the addition of four molecules of water, $NH_4OOC \cdot COONH_4 = NC—CN + 4H_2O$. Cyanogen is thus proved by these reactions to be the nitrile of oxalic acid, the constitutional formulae of which are:



$= N \equiv C - C \equiv N$

In consideration of these chemical relationships, the mutual occurrence of cyanogen and calcium oxalate in plant cells may not be unusual from a chemical standpoint.

The Taxonomic and Climatic Distribution of Oxalate Families

According to Berger (1908), Frey (1925), Kraemer (1907), Kunth (1924), Limpricht (1928), Solereder (1908, 1928), Warburg (1900), and Winton (1916), calcium oxalate is found in 215 plant families. These families are listed in table 2 and are classified climatically by Engler and Gilg (1919), and Willis (1925). An analysis of their data shows that 118, or 54.13 percent, are mostly tropical; 6, or 2.75 percent, are mostly tropical-subtropical; 3, or 1.37 percent, are mostly subtropical; 5, or 2.29 percent, are subtropical-temperate; 39, or 17.89 percent are temperate; and 47, or 21.56 percent, are widely distributed in habitat.

The climatic distribution of oxalate families may be compared with the climatic distribution of the total number of plant families. Making use again of the climatic distribution of these by Engler and Gilg and Willis it becomes apparent that of the 295 families of gymnosperms and angiosperms 218, or 73.90 percent, contain oxalate; of 153 tropical families 118, or 77.12 percent, have oxalate; of 15 tropical-subtropical families 6, or 40.00 percent, have oxalate; of 8 subtropical families 3, or 37.50 percent, have oxalate; of 7 subtropical-temperate families 5, or 71.43 percent, have oxalate; of 50 temperate families 39, or 78.00 percent, have oxalate, and of 57 families of widely distributed habitats 47, or 82.46 percent, have oxalate.

It is evident that of the plant families producing oxalate nearly three times as many are found in the tropics as in the temperate zone. Those mostly tropical in distribution include 118, or 54.13 percent, of all the oxalate

TABLE 2. *The Taxonomic and Climatic Distribution of Cyanogenetic Glucosides and Calcium Oxalate*

Family	Cyanogenetic Glucosides	Ordinary Crystals	Clustered Crystals	Rhombohedral Solitary Crystals	Spherulites	Octahedral or Prismatic	Raphide Bundles	Typical Styloids	Resembling Styloids	Typical Crystal Sand	Fine Sand
Acanthaceae‡		×				×					
Aceraceae*		×	×		×	×		×		×	
Aizoaceae‡		×				×	×		×		
Amarantaceae‡ †		×									
Anacardiaceae‡	×	×				×		×			×
Anonaceae‡		×				×		×			
Apocynaceae‡		×									
Aquifoliaceae§		×				×		×			
Araceae‡	×	×				×					
Araliaceae‡		×				×				×	
Aristolochiaceae§		×				×					×
Asclepiadaceae‡	×	×			×			×			
Balanophoraceae‡		×			×						
Balanopsidaceae‡		×				×					
Basellaceae‡		×				×					
Batidaceae‡ †		×				×					
Begoniaceae‡		×	×		×						
Berberidaceae*	×	×				×					
Betulaceae*		×				×					
Bignoniaceae‡		×				×					
Bixaceae‡		×				×					
Boraginaceae§		×	×			×					
Bromeliaceae‡	×	×				×					
Bruniaceae‡		×				×					
Burmanniaceae‡		×				×					
Burseraceae‡		×				×					
Buxaceae§		×				×					
Cactaceae*		×			×	×			×	×	
Calycanthaceae§	×		×			×					
Calyceraceae*			×			×					
Campanulaceae† *			×			×					
Cannaceae‡		×		×		×		×		×	
Capparidaceae‡		×			×						
Caprifoliaceae*	×	×				×		×			
Caricaceae‡		×	×		×						×
Caryophyllaceae*		×	×			×			×		
Casuarinaceae‡		×				×					
Celastraceae§	×	×	×			×			×		
Chenopodiaceae§		×				×					
Chlaenaceae‡		×	×			×			×		
Chloranthaceae‡		×				×					
Cistaceae*		×				×					
Cochlospermaceae‡		×				×			×		
Columelliaceae*		×				×					
Combretaceae‡		×				×					
Commelinaceae‡	×	×				×			×		
Compositae§	×	×				×					
Connaraceae‡		×				×					
Convolvulaceae‡	×	×				×					
Coriariaceae*		×				×					
Cornaceae§	×	×				×					
Corynocarpaceae‡		×				×					

TABLE 2.—*Continued*

Family	Cyanogenetic Glucosides	Ordinary Crystals	Clustered Crystals	Rhombohedral Solitary Crystals	Spherulites	Octahedral or Prismatic	Raphide Bundles	Typical Styloids	Resembling Styloids	Typical Crystal Sand	Fine Sand
Crassulaceae§			×	×	×						
Cruciferae*	×									×	
Cucurbitaceae‡				×							
Cyclanthaceae‡		×	×					×	×		
Cynocrambaceae† *							×				
Cyrillaceae§		×									
Datiscaceae§						×					
Diapensiaceae*		×									
Dichopetalaceae‡		×									
Dilleniaceae‡							×				
Dioscoreaceae‡								×			
Dipsacaceae§			×								
Dipterocarpaceae‡		×	×								
Droseraceae§	×										
Ebenaceae‡		×									
Elaeagnaceae§						×					
Elatinaceae§			×								
Empetraceae*			×		×						
Epacridaceae‡		×									
Ericaceae§		×									
Eriocaulaceae‡		×	×			×					
Euphorbiaceae§	×	×	×		×			×	×		
Fagaceae§		×									
Flacourtiaceae‡	×	×									
Flagellariaceae‡			×								
Fouquieriaceae‡					×						
Frankeniaceae§			×								
Gentianaceae*			×			×					
Geissolomataceae‡		×									
Geraniaceae‡ †		×			×		×	×			
Gesneriaceae‡			×			×	×				
Globulariaceae*						×					
Gomortegaceae*						×					
Gonystilaceae‡		×									
Goodeniaceae‡						×					
Gramineae§	×										
Grubbiaceae‡		×									
Guttiferae‡		×				×					
Haloragaceae§			×								
Hamamelidaceae†		×									
Hernandiaceae‡				×		×					
Hippocastanaceae§		×									
Hippocrateaceae‡		×									
Humiriaceae‡		×									
Hydnoraceae‡						×					
Hydrophyllaceae*			×								
Icacinaceae‡		×								×	
Juglandaceae*		×							×		
Labiatae§		×	×			×					
Lacistemaceae‡		×									
Lardizabalaceae*					×						
Lauraceae‡	×					×					
Lecythidaceae‡		×									
Leguminosae§	×	×		×	×	×			×		

TABLE 2.—*Continued*

Family	Cyanogenetic Glucosides	Ordinary Crystals	Clustered Crystals	Rhomboliedral Solitary Crystals	Spherulites	Octahedral or Prismatic	Raphide Bundles	Typical Styloids	Resembling Styloids	Typical Crystal Sand	Fine Sand
Leitneriaceae*			×								
Lemnaceae§			×				×				
Lentibulariaceae§											
Liliaceae§		×				×					
Linaceae§	×	×				×	×				
Loasaceae§			×								
Loganiaceae‡		×	×			×		×	×	×	
Loranthaceae‡		×									
Lythraceae§		×			×	×		×			
Magnoliaceae‡ †	×		×			×					
Malpighiaceae‡		×							×		
Malvaceae§		×		×		×					
Marantaceae‡		×	×		×	×		×			
Melastomataceae‡	×						×				
Meliaceae‡		×				×					
Melianthaceae‡			×								
Menispermaceae‡		×				×					
Monimiaceae‡											
Moraceae‡		×	×			×					
Moringaceae‡		×									
Musaceae‡			×								
Myoporaceae‡	×	×	×			×					
Myricaceae†		×									
Myristicaceae‡			×			×					
Myrothamnaceae‡			×								
Myrsinaceae‡		×				×					
Myrtaceae‡		×									
Myzodendraceae*											
Nepenthaceae‡			×								
Nolanaceae‡			×			×	×	×	×	×	
Nyctaginaceae‡			×								
Nymphaeaceae† *			×								
Ochnaceae‡		×	×								
Octoknemataceae‡				×							
Olacaceae‡	×	×									
Oleaceae§						×					
Onagraceae§			×			×	×	×			
Orchidaceae‡			×	×							
Papaveraceae*	×		×								
Palmae‡		×	×								
Passifloraceae‡	×	×									
Pandanaceae‡		×									
Pedaliaceae‡		×				×					
Penaeaceae‡			×								
Pentaphylacaceae*		×									
Philydraceae‡			×		×	×	×	×	×		
Phytolaccaceae‡		×				×					
Piperaceae‡		×					×				
Pittosporaceae‡		×									
Platanaceae*	×	×									
Plumbaginaceae§		×									
Podostemaceae‡											
Polemoniaceae*			×		×						

TABLE 2.—*Continued*

Family	Cyanogenetic Glucosides	Ordinary Crystals	Clustered Crystals	Rhombohedral Solitary Crystals	Spherulites	Octahedral or Prismatic	Raphide Bundles	Typical Styloids	Resembling Styloids	Typical Crystal Sand	Fine Sand
Polygalaceae§		×									
Polygonaceae*		×									
Pontederiaceae‡			×					×	×		
Portulacaceae§		×									
Proteaceae‡	×	×									
Rafflesiaceae‡						×					
Ranunculaceae*	×		×			×					
Resedaceae*		×					×				
Restionaceae† *		×						×			
Rhamnaceae§		×									
Rhizophoraceae‡		×									
Rosaceae§	×	×			×						
Rubiaceae‡	×	×			×	×	×	×	×	×	
Rutaceae§		×					×	×		×	
Sabiaceae‡		×			×						
Salicaceae*		×									
Santalaceae§		×									
Sapindaceae‡	×	×						×	×	×	
Sapotaceae‡	×	×								×	
Sarraceniaceae*			×								
Saxifragaceae*	×	×				×	×	×		×	
Scheuchzeriaceae*	×										
Scrophulariaceae§	×	×				×					
Scytopetalaceae‡				×							
Simarubaceae‡		×				×					
Solanaceae§		×			×	×		×		×	
Staphyleaceae‡		×									
Sterculiaceae‡	×	×				×					
Stylidiaceae‡			×								
Styracaceae§		×				×					
Taccaceae‡			×								
Tamaricaceae† *		×									
Theaceae‡ †		×					×	×			
Thymelaeaceae§		×	×					×	×	×	
Tiliaceae‡	×	×							×		
Tremandraceae‡		×									
Trochodendraceae*			×								
Turneraceae‡		×							×		
Ulmaceae§		×									
Umbelliferae*		×					×	×			
Urticaceae‡	×	×									
Valerianaceae§			×			×					
Verbenaceae‡		×				×	×				
Vitaceae‡		×				×					
Vochysiaceae‡		×									
Winteranaceae‡	×	×				×					
Xyridaceae‡		×	×								
Zygophyllaceae‡		×				×	×	×	×		
Zingiberaceae‡					×						×

‡ Mostly tropical
‡ † Mostly tropical-subtropical
† Mostly subtropical
† * Mostly subtropical-temperate
* Mostly temperate
§ Widely distributed

NOTE: Additional oxalate families to those tabulated above are: Crossosomataceae(*), Droseraceae (§), Lennoaceae(†), Orobanchaceae(*), Plantaginaceae(*), Primulaceae (*), Pyrolaceae (*), Salvadoraceae (‡), Stackhousiaceae(‡). Cycadaceae, (‡) Gnetaceae (‡ †), Gramineae (§), and Pinaceae (*).

families while only 39, or 17.89 percent, are found growing largely in the temperate zone. The ratio of tropical oxalate families to the total tropical families (77.12 percent), however, is about equal to the ratio of temperate oxalate families to the total temperate families (78.00 percent).

Climatic Distribution of Oxalate Crystal Types

The climatic distribution of the various crystal types in which calcium oxalate is precipitated in plants is shown in table 3. These different crystal forms and aggregates that have been distinguished in these families are likewise shown in table 2. They are: (1) *ordinary solitary crystals*, of large but variable size, which are very widely distributed together with their modifications and twin crystals; (2) *styloids* or columnar crystals or twin forms of such crystals, the latter when typically differentiated reminding one of the characteristic crystals of gypsum; styloids commonly replace raphides in plants and occasionally show transitions to the form of crystals, discussed under (4); (3) *octahedral or prismatic crystals* generally of rather small size; (4) *acicular crystals*, *i.e.*, small needle-shaped or fusiform crystals which sometimes merge into small crystals of prismatic shape, or crystals which are almost of the nature of styloids; when elongated, the acicular crystals may resemble raphides, but when they are very small they look like sand; in most cases large numbers of acicular crystals occur in the same cell; (5) *clustered crystals;* (6) *spherulites*, spherical concretions of clustered crystals with a smooth or rather smooth surface; (7) *raphides*, *i.e.* long acicular crystals, large numbers of which lie parallel to one another in a mucilage-containing cell; (8) *crystal sand* composed of many small crystals which completely fill the cells.

Of the 391 known recorded familial occurrences of the various forms of oxalate, ordinary crystals occupy 135, or 34.52 percent; octahedral or prismatic crystals 68, or 17.40 percent; clustered crystals 59, or 15.08 percent; typical styloids 31, or 7.93 percent; spherulites 21, or 5.37 percent; styloid-resembling 18, or 4.60 percent; typical crystal sand 20, or 5.11 percent; raphide bundles 29, or 7.42 percent; and rhombohedral solitary crystals 10, or 2.55 percent.

Several forms of crystals may occasionally occur side by side in the same plant, the chief combinations of which are solitary and clustered crystals, raphides and clustered crystals, raphides and styloids, clustered crystals and crystal sand (the clustered crystals are sometimes even enclosed in the cells that contain the crystal sand). In these cases the various forms of crystals are found either in the same or in different tissues, or even in different organs of the plant. In other species or genera, or even orders, however, only one form of crystal is present. It must be borne in mind that this salt has been capable of re-entering into the metabolism of the plant and that clustered crystals often occur alone in the primary cortex in early stages, while solitary crystals appear only subsequently.

TABLE 3. *The Climatic Distribution of Oxalate Crystal Types*

	Ordinary		Clustered		Rhombohedral		Spherulites		Octahedral		Raphide Bundles		Typical Styloids		Resembling Styloids		Crystal Sand	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Tropical	79	58.09	32	54.24	8	80.00	8	38.09	39	57.35	21	72.41	19	61.29	9	50.00	10	50.00
Tropical-subtropical	4	2.94	1	1.70	0	0	1	4.76	1	1.67	2	6.90	2	6.45	0	0	1	5.00
Subtropical	2	1.47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subtropical-temperate	2	1.47	2	3.39	0	0	0	0	2	2.94	1	3.45	0	0	0	0	0	0
Temperate	19	13.97	10	16.95	0	0	6	28.57	9	13.23	1	6.90	2	6.45	2	11.11	2	10.00
Widely distributed	30	22.06	14	23.73	2	20.00	6	28.57	17	25.00	4	13.79	8	25.80	7	38.88	7	35.00

Mono- and Tri-hydrate Oxalate

Occurrence

Calcium oxalate occurs in two chemical forms, the monohydrate $CaC_2O_4 \cdot H_2O$ and the trihydrate, $CaC_2O_4 \cdot 3H_2O$. Each one of these has a different crystal structure, the monohydrate belonging to the monoclinic system of crystallization and the trihydrate to the tetragonal system. In the monoclinic system there are three crystallographic axes unequal in length, with one of their intersections oblique, and the two other intersections equal to 90°. The tetragonal system has three axes at right angles to each other, two of them (the lateral axes) equal, the third (the vertical axis) longer or shorter. The oxalate crystal types described previously in this paper and tabulated in table 2 may therefore be reclassified according to the monoclinic and tetragonal systems. Ordinary crystals, clustered crystals, spherulites, raphide bundles, styloids, and crystal sand may belong to either the monoclinic or the tetragonal systems.

The crystallization of calcium oxalate monohydrate is monoclinic. Four different surface forms are present in these plant crystals *viz.*, two pinacoid, and two prisms. These four forms combine to produce two different typical combinations. One of the forms of the monoclinic crystals comprises a combination of a prism with a pinacoid to form the well known isodiametrical crystal. This crystal when remote from its ideal form is like a rhomboid. As a consequence in the old literature the crystal system of the monohydrate was erroneously designated as rhombic or orthorhombic. The group termed "rhombohedral" in tables 2 and 3 is therefore monoclinic. The two pinacoids may appear together in which case the crystals are usually extended and develop into styloids. The size of the crystal cell determined whether the prismatically extended styloid or the isodiametric crystal formed. The trihydrate crystallizes tetragonally. The crystals in cross-section and in general appearance are squares. Only three face forms have been noticed up to the present time; they are the bipyramid, the pyramid, and the prism. The most frequent type of face is the bipyramid, which gives the crystals the well known "envelope" appearance. Usually the pyramid combines with the prism to allow the crystal to develop as a styloid. The trihydrate may be distinguished easily from the monohydrate by means of the bipyramid and the absence of a sharp angle. The group designated "octahedral and prismatic" in tables 1 and 2 is therefore of the trihydrate oxalate with a tetragonal crystallization.

Climatic Distribution

Frey (1929) has determined that calcium oxalate monohydrate forms at temperatures from 0° to 100° C. in a solution of any vapor pressure or any osmotic pressure. It is absolutely stable and permanent in the above range of formation. He also found out that calcium oxalate trihydrate forms at temperatures from 0° to about 30° C. in solutions with high vapor pressure

(low osmotic pressure) and only when saturated with calcium oxalate. The trihydrate is metastable in the specified range of formation and has a tendency to change to the monohydrate. In solutions the permanence of the trihydrate is maintained by the presence of calcium ions and high viscosity, but diminished by H^+ and $C_2O_4^{--}$ ions.

From the conditions required for formation, stability, and permanence of the two calcium oxalates it might be expected that the trihydrate might exist more readily in plants in temperate climates and the monohydrate be more abundant in tropical plants.

Table 3 shows the climatic distribution of the various oxalate crystal types. The column headed "rhombohedral" gives the distribution of the monohydrate and the heading "octahedral" gives the corresponding occurrence of the trihydrate. From a consideration of these it is evident that the monohydrate is mainly tropical and the trihydrate well distributed in both tropical and temperate climates. These anatomical findings in vivo therefore correspond to the chemical findings in vitro.

In this connection it is interesting to note that calcium oxalate monohydrate occurs in small colorless monoclinic crystals as the mineral "whewellite" in coal. This might indicate a tropical climate existing at the time of coal formation.

Mutual Distribution of Oxalate and Cyanogen

Cyanogen is known to occur in plants as free hydrogen cyanide and combined in cyanogenetic glucosides. The 39 families found to contain cyanogenetic glucosides (McNair, 1932) are listed in table 2. Of these families only 1 (Scheuzeriaceae), or 2.56 percent, does not produce oxalate.

Conclusions

The knowledge that cyanogen may be changed chemically to oxalic acid and that oxalic acid may be changed into cyanogen coupled with the fact that 97.44 percent of the cyanogenetic glucoside plant families also produce oxalic acid lends credence to the possibility of transformation of cyanogen to oxalic acid in plants.

The fact that calcium oxalate is capable of re-entering the metabolism of the plant and that clustered crystals often occur alone in the primary cortex in early stages, while solitary crystals appear only subsequently, lends a degree of probability to the hypothesis of a change of oxalic acid to cyanogen in the plant, for calcium oxalate is practically insoluble in water (one hundred parts of water dissolve 0.000554 gm. at 18° C. and 0.0014 gm. at 95° C.). However, as cyanogenetic glucosides have been located in only 39 out of a total of 218 oxalate families, or 17.89 percent, it seems quite likely that oxalic acid may be formed in plants from some other substances than cyanogen unless in these instances all the available cyanogen is used up in the reaction.

Summary

Essential oils, resins, and calcium oxalate all occur in twice as many tropical families as temperate.

Essential Oils and Resins

Essential oils are found in 87, or 29 percent, of the 295 families of higher plants listed by Engler and Gilg. Of the essential oil families 44 percent are tropical, 19 percent are temperate, and 27 percent, widely distributed.

Resins are found in appreciable amounts in 26 families, or 9 percent of the higher plant families. Of the resin families 50 percent are tropical, 23 percent are temperate, and 23 percent are widely distributed.

Of the essential oil families, 28 percent have resin in appreciable quantity, while 100 percent of the resin families possess essential oils.

There is a close parallelism between the percentages of essential oil and resin families in similar climatic habitats.

Both substances are located in similar, and sometimes share the same anatomical structures.

Essential oils contain substances which form resins either by condensation, polymerization, or both.

Among the functions of essential oils may be those of solvents for, transporting agents for, and mother substances of, resins. As essential oil and resin families occur both in the tropics and temperate zone they should be useful in both, but are probably more useful in the tropics on account of their greater occurrence in that habitat.

Cyanogen and Oxalate

The simultaneous occurrence of prussic acid, protein matter, and calcium oxalate has been noted in cells in *Pangium edule*.

Oxalic acid is chemically related to cyanogen because: (1) ammonium oxalate when heated with a dehydrator produces cyanogen, and (2) if cyanogen be dissolved in hydrochloric acid, ammonium oxalate is formed.

Calcium oxalate is found in 218 plant families of which 54 percent are mostly tropical; 3 percent are tropical-subtropical; 1 percent is subtropical; 2 percent are subtropical-temperate; 18 percent are temperate; and 21 percent are widely distributed in habitat.

Oxalate of lime is deposited in plants in 8 different crystal types. The percentage distribution of these in plant families is given. The various combinations of mixed crystals that occur in plants are likewise given.

Calcium oxalate occurs in two chemical forms, the monohydrate $CaC_2O_4 \cdot H_2O$ and the trihydrate $CaC_2O_4 \cdot 3H_2O$. The monohydrate is monoclinic and the trihydrate is tetragonal.

From the conditions required for formation, stability, and permanence of the two calcium oxalates it might be expected that the trihydrate might

exist more readily in plants in temperate climates and the monohydrate be more abundant in tropical plants. The findings in plants correspond to the findings in the chemical laboratory in this respect.

The presence of the monohydrate in coal might indicate a tropical climate existing at the time of coal formation.

Cyanogenetic glucosides have been discovered in 39 families. Of these families only 1, or 2.5 percent, does not produce oxalate.

Oxalate of lime is practically insoluble in water and yet re-enters the metabolism of the plant. Consequently some chemical change may occur, perhaps transformation into cyanogen.

Cyanogenetic glucosides have been located in only 39 out of a total of 218 oxalate families, or 18 percent. It seems likely, therefore, that oxalic acid may be formed in plants from some other substance than cyanogen unless in these instances all the available cyanogen is used up in the reaction.

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INHERITANCE OF RESISTANCE TO LOOSE AND COVERED SMUT IN HYBRIDS OF HULL-LESS WITH EARLY GOTHLAND AND MONARCH OATS[1]

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I have recently published two papers describing some of my results on the investigation of smut resistance in certain oat hybrids. One of these (17) dealt with hybrids of Early Gothland and Monarch and the other (18) recorded the results obtained with a hybrid between Early Gothland and Victor. The data secured with the Missouri races of both loose (*Ustilago Avenae* (Pers.) Jens.) and covered (*U. levis* (K. & S.) Magn.) smuts were included. The Early Gothland variety is characterized by its high susceptibility to the loose smut and a corresponding resistance to the covered. Monarch is exactly the reverse in its behavior, being extremely susceptible to the covered smut and resistant to the loose. The Victor oat differs from both of these in being completely susceptible to loose and covered smut.

The present paper is concerned with the results obtained with hybrids between Early Gothland and Hull-less and Monarch and Hull-less. Hull-less is an oat variety which is very susceptible to the Missouri races of both loose and covered smut. It has been used in a great many different types of experiments and high percentages of infection have usually been obtained (8, 9, 11, 15, 20). In connection with the hybrids grown in the present series, as recorded in table 1, there were 237 plants of Hull-less inoculated with the loose smut and 206 (86.9 percent) were infected. In the series with covered smut 185 plants were inoculated and 148 (80 percent) were infected. In some of the individual experiments this variety has given 100 percent infection; in other experiments, however, one or more plants have failed to be smutted. It is interesting to note that Hull-less has, on the average, given lower percentages of infection than Victor, Canadian, and some other oat varieties. However, it is certainly to be classified as a very susceptible oat.

Along with the hybrids, the two varieties, Early Gothland and Monarch, were also grown for direct comparison. As recorded in table 1, Early Gothland gave 100 percent infection with the loose smut, 62 plants being inoculated. At the same time, it was comparatively free from infection with the covered smut, only 3 plants out of a total of 147 (2 percent) being recorded as infected. The Monarch variety was also inoculated with the loose smut, but only 2 plants out of a total of 208 (0.9 percent) were infected. With the

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covered smut, however, this variety gave 100 percent infection, 78 plants being inoculated.

The crosses between Early Gothland and Hull-less and Monarch and Hull-less were made in 1925, the first generation plants being grown in the greenhouse in 1926. Two crosses between Early Gothland and Hull-less were made, in both cases Early Gothland being used as the female parent. There were four crosses between Monarch and Hull-less; in one of these (Hybrid 24) Monarch was used as the female parent and in the other three (Hybrids 25–27) Hull-less was used as the female parent.

Early Gothland and Monarch are both characteristic hulled oats. The most obvious difference between the two varieties is the color of the glumes or hulls of the grain, those of Early Gothland being white or pale yellow, while those of Monarch are dark in color. Hull-less, on the other hand, is a typical naked oat. The empty glumes and lemmas are very similar in texture, being thin and membranous; they do not clasp the kernel, the latter remaining loose or free within, and readily separating in threshing. Each spikelet of the panicle bears several flowers, varying from four to nine. The rhachillas of the spikelets are much elongated so that the uppermost grains are borne well above the empty glumes.

The F_1 plants of Early Gothland $\times$ Hull-less showed the dominance of the many-flowered, naked seed character of the male parent. Most of the spikelets were many-flowered, the number of grains produced being from two to five. The empty glumes and lemmas were quite similar to those of the Hull-less parent, and the grains were held loosely in the glumes. In a few spikelets, however, the lemma had developed a hard ridge on the back, the kernels being held more or less securely within the glumes.

The F_1 plants of Hybrids 24–27 were also similar to the Hull-less parent. Most of the spikelets were many-flowered, and the grain easily separated from the glumes. There were a few spikelets in which the grains were held somewhat securely between the hardened glumes. All of these F_1 plants, however, differed from those between Early Gothland and Hull-less in that the palea was dark colored, a character inherited from the Monarch parent.

Methods of Inoculation

It is important to emphasize the effectiveness of my methods of inoculation. The procedure has involved several different things. It has been clearly demonstrated by several investigators, Bartholomew and Jones (1), Reed and Faris (19), Sampson (23, 24), von Rosenstiel (22), Stanton, Coffman, Tapke, Wiebe, Smith and Bayles (25), and Nicolaisen (7), that environal factors play a very important part in the successful infection of susceptible oats with either loose or covered smut. A susceptible variety may readily fail to be infected with a virulent smut solely on account of adverse environal conditions. Our extensive investigations have shown that the following conditions are important:

1. The substratum. Sand has proved an ideal substratum for the germination of the oat seedlings. It is easily cleaned, graded, and later separated from the young plants. Further, a very high percentage of infection of susceptible varieties may be obtained in this substratum.

2. The moisture content of the substratum must be controlled, a matter which is easily done with sand. We have found that a low moisture content is most effective for successful infection, although the range is comparatively wide. The best and surest results are secured when the sand moisture is approximately 20 percent of its water-holding capacity.

3. The temperature of the substratum at which the seed is germinated is also an important factor. While there is a rather wide range of temperature for infection, in general the best results are secured if the seed is germinated at approximately 20° C.

4. Removal of the hulls. In all of my investigations on the inheritance of smut resistance and also those on physiologic specialization, the hulls have been removed from the caryopsis. This method was originally adopted in the case of the hybrids of Hull-less × Black Mesdag, since some of the F_2 plants were characteristically hulled types, while others were hull-less. The hulls from the former were removed in order to give the different progenies a uniform chance, so far at least, as hulls were concerned. In many of my experiments the presence or absence of the hulls apparently made no appreciable difference in the percentage of infection. In other experiments, however, the presence of the hulls has reduced somewhat the number of infected plants. They are to be regarded as one more factor in environal conditions which can easily be controlled.

Germinating the inoculated seed under these controlled conditions, of course, has involved the necessity of transplanting the young seedlings. This can be done quite successfully in five to seven days after the seed is placed in the germinator. The seedlings are easily handled and, if ordinary care is used, there is no appreciable loss. Following the germination of the seedlings, there is no apparent difference in the percentage of smutted plants depending upon whether they are grown to maturity in the greenhouse or in the field. The results of our experiments certainly justify such careful attention to methods, since sowing the seed in the open under varied field conditions has not given particularly impressive results.

In all the studies herein reported the Missouri races of both *Ustilago Avenae* and *U. levis* have been used for inoculating the parents and hybrids. The parental varieties are also definite strains which I have grown in my various investigations, their seed numbers being recorded in table 1.

Hybrids of Early Gothland and Hull-less

I have previously described some of the results secured with Hybrids 19 and 20, Early Gothland × Hull-less (12, 13, 14, 16). The first set of second generation plants was grown in the greenhouse in 1927. There were three

series of these F_2 plants, one being inoculated with *Ustilago Avenae*, a second being inoculated with *U. levis*, and a third consisting of uninoculated plants. Additional F_2 plants were grown to maturity in the field in 1928, some being inoculated with loose smut and others with the covered smut.

Data Obtained with the F_2 Generation

Results with Ustilago Avenae. We have already mentioned the data obtained with the two parents, Early Gothland and Hull-less, and recorded in table 1. The data indicate clearly that both parents are extremely

TABLE 1. *Results with Inoculated F_2 Plants of Crosses between Early Gothland and Hull-less and between Monarch and Hull-less*

		Inoculated with Ustilago Avenae			Inoculated with Ustilago levis		
	Date Grown	No. Plants	No. Inf.	Per-cent Inf.	No. Plants	No. Inf.	Per-cent Inf.
Hybrid 19 Early Gothland × Hull-less.....	1927	24	22	91.6	49	18	36.7
	1928	15	14	93.3	55	31	56.3
		39	36	92.3	104	49	47.1
Hybrid 20 Early Gothland × Hull-less.....	1927	22	21	95.4	45	19	42.2
	1928	29	28	96.5	42	20	47.6
		51	49	96.0	87	39	44.8
Total, Hybrids 19, 20.....		90	85	94.4	191	88	46.0
Hybrid 24 Monarch × Hull-less.....	1927	49	12	24.4	25	22	88.0
	1928	53	22	41.5	12	12	100.0
		102	34	33.3	37	34	91.8
Hybrid 25 Hull-less × Monarch.....	1927	50	11	22.0	27	27	100.0
	1928	55	24	43.6	13	13	100.0
		105	35	33.3	40	40	100.0
Hybrid 26 Hull-less × Monarch...	1927	48	7	14.5	23	22	95.6
	1928	54	21	38.8	11	11	100.0
		102	28	27.4	34	33	97.0
Hybrid 27 Hull-less × Monarch.....	1927	49	11	22.4	25	24	96.0
	1928	57	22	38.5	12	12	100.0
		106	33	31.1	37	36	97.2
Total, Hybrids 24–27.....		415	130	31.3	148	143	96.6
Hull-less (30)*.....		237	206	86.9	185	148	80.0
Early Gothland (152).....		62	62	100.0	147	3	2.0
Monarch (161).....		208	2	0.9	78	78	100.0

* The number in parenthesis after the varietal name is the seed number of the strain used.

All the plants in 1927 were grown in the greenhouse and those for 1928 in the field.

susceptible to the loose smut. In 1927 there were inoculated 24 F_2 plants of Hybrid 19, of which 22 (91.6 percent) were infected; at the same time, 22 F_2 plants of Hybrid 20 were inoculated and 21 (95.4 percent) were infected. The additional inoculated plants of both hybrids grown in 1928 gave corresponding high percentages of infection. Altogether, there were 90 F_2 plants of the two hybrids inoculated with the loose smut and 85 (94.4 percent) were infected. The results indicate very clearly that the second generation of these hybrids is susceptible like the parents. The 5 plants which failed to become infected may be regarded as casual escapes.

Results with Ustilago levis. The first data with this smut were also obtained in 1927. There were inoculated 49 plants of Hybrid 19, of which 18 (36.7 percent) were infected; 45 plants of Hybrid 20 were inoculated and 19 (42.2 percent) were infected. Additional inoculated plants of both hybrids were grown to maturity in the field in 1928 and with both hybrids somewhat higher percentages of infection were secured as compared with the previous year. There were 55 plants of Hybrid 19 inoculated, of which 31 (56.3 percent) were infected, and of Hybrid 20 there were 42 plants of which 20 (47.6 percent) were infected. Altogether, of the two hybrids there were grown 191 F_2 plants inoculated with the covered smut and 88 (46 percent) were smutted.

These results are interesting on account of the relatively large number of infected second generation plants. In all of the crosses which I have previously reported, the percentage of infected F_2 plants approximated 25 percent of the total, in practically every case, however, falling below that percentage. With these two hybrids between Early Gothland and Hull-less, however, there is a close approach to 50 percent infection among the F_2 plants.

Data Obtained with the F_3 Generation

In addition to the second generation plants inoculated with loose and covered smut, a number of uninoculated F_2 plants were also grown. Accordingly, there were available for the study of smut resistance in the third generation three classes of progenies: (1) descended from the uninoculated second generation plants; (2) descended from F_2 plants which had been inoculated with *Ustilago levis* and survived; and (3) descended from F_2 plants which had been inoculated with *U. Avenae* and survived. In tabulating the results obtained with these progenies, they are separated into three groups on the basis of the amount of infection obtained: (1) resistant, progenies in which no infected plants were observed; (2) segregating, progenies in which the percentage of infection was less than 50 percent; and (3) susceptible, progenies in which the percentage of infection exceeded 50 percent.

Results with Ustilago Avenae. In view of the high susceptibility of the second generation plants to the loose smut, relatively few third generation progenies were inoculated with it, the data being recorded in table 2. There

TABLE 2. *Results with the F_3 Progenies of Hybrids 19, 20, Early Gothland × Hull-less, Inoculated with Ustilago Avenae*

Hybrid and Progeny Number	Total No. Progenies	Total No. Plants	No. Inf.	Percent Inf.
Descended from uninoculated F_2 plants				
Hybrid 19-F_3-1, 3, 5, 7, 8, 9, 10, 11, 12, 13, 15, 16.....	12	229	229	100.0
Hybrid 20-F_3-1, 2, 3, 4, 5, 7, 11, 12, 13, 14, 15, 16.....	12	226	226	100.0
	24	455	455	100.0
Descended from F_2 plants inoculated with Ustilago levis				
Hybrid 19-F_3- 101, 102, 103, 105, 107, 109, 112, 116, 120, 123, 125, 127.....	12	235	235	100.0
Hybrid 20-F_3- 101, 102, 103, 109, 110, 112, 114, 117, 119, 122, 124, 126.....	12	225	222	98.6
	24	460	457	99.3
Descended from F_2 plants inoculated with Ustilago Avenae				
Hybrid 19-F_3- 51, 52, 53.....	3	40	39	97.5
Hybrid 20-F_3- 51.....	1	13	11	84.6
	4	53	50	94.3

were grown 12 progenies of each of the two hybrids which had descended from the uninoculated F_2 plants; these 24 progenies contained 455 individuals, every one of which was infected. There were also grown 12 progenies of each hybrid descended from the F_2 plants which had been inoculated with covered smut; they contained 460 plants, of which 457 (99.3 percent) were infected. As noted above, 5 second generation plants survived inoculation with the loose smut, and progenies from 4 of these were grown in the following generation. Altogether, 53 plants belonging to these 4 progenies were grown to maturity and 50 of them (94.3 percent) were infected. The evidence, then, is quite clear that the F_2 plants of these progenies merely escaped infection with the loose smut.

The results indicate a high degree of susceptibility of the third generation progenies of these hybrids to the loose smut, and they correspond very closely to those obtained with the second generation plants.

Results with Ustilago levis. In view of the interesting behavior of the inoculated second generation plants to the covered smut, a large number of third generation progenies were inoculated with it. There were 46 progenies of the two hybrids descended from uninoculated F_2 plants, the data for which are recorded in table 3 and summarized in table 5. They are arranged on the basis of their behavior—resistant, segregating, or susceptible.

TABLE 3. *Results with the F_3 Progenies of Hybrids 19, 20, Early Gothland × Hull-less, Descended from Uninoculated F_2 Plants. All Progenies Inoculated with Ustilago levis*

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
Resistant F_3 families							
19-F_3- 3	44	0	0	20-F_3- 13	42	0	0
18	25	0	0				
19	24	0	0				
Segregating F_3 families							
19-F_3- 5	39	9	23.0	19-F_3- 15	42	7	16.6
7	44	8	18.1	17	25	6	24.0
9	41	20	48.7	20	22	5	22.7
11	43	17	39.5	21	25	8	32.0
12	43	12	27.9				
20-F_3- 1	33	12	36.3	20-F_3- 14	42	1	2.3
4	36	14	38.8	16	42	13	30.9
8	22	4	18.1	19	20	1	5.0
12	39	16	41.0	21	23	8	34.7
Susceptible F_3 families							
19-F_3- 1	44	43	97.7	19-F_3- 13	41	37	90.2
2	22	22	100.0	14	23	13	56.5
4	24	16	66.6	16	42	38	90.4
6	21	16	76.1	22	24	20	83.3
8	41	32	78.0	23	25	25	100.0
10	19	10	56.2	24	25	17	68.0
20-F_3- 2	44	31	70.4	20-F_3- 11	43	40	93.0
3	42	38	90.4	15	39	36	92.3
5	37	24	64.8	17	20	18	90.0
6	22	22	100.0	20	21	20	95.2
7	39	33	84.6	22	25	25	100.0
9	21	14	66.6	23	22	20	90.9
10	20	16	80.0				

Of the 46 progenies, 3 belonging to Hybrid 19 and 1 to Hybrid 20 gave no infection, a total of 135 plants being grown. There were 17 progenies which were classified as segregating, the range of infection varying from 2.3 to 48.7 percent. Of these progenies 9 belonged to Hybrid 19 and 8 to Hybrid 20. Altogether, they contained 581 plants of which 161 (27.7 percent) were infected, the percentages of infection obtained for the two hybrids corresponding quite closely. Finally, there were 25 families which were classified as susceptible. Thus, more than half of the total number of progenies grown belonged in this group. The lowest percentage of infection obtained was 56.2 percent; 4 of the progenies gave 100 percent infection, and 9 additional ones gave percentages above 90 percent. There can be no question regarding the high degree of susceptibility of most of these families.

TABLE 4. *Results with the F_3 Progenies of Hybrids 19, 20, Early Gothland × Hull-less, Descended from F_2 Plants which had been Inoculated with Ustilago levis. All Progenies Inoculated with Ustilago levis*

Resistant F_3 families

Hybrid and Progeny Number	Total No. Progenies	Total No. Plants	No. Inf.	Percent Inf.
Hybrid 19-F_3- 110, 118, 127, 128, 131, 145, 148	7	183	0	0
Hybrid 20-F_3-102, 106, 112, 115, 121, 122, 126, 131, 132, 136, 144	11	309	0	0
	18	492	0	0

Segregating F_3 families

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
19-F_3- 102	43	18	41.8	19-F_3- 130	25	1	4.0
103	42	14	33.3	132	23	2	8.6
104	21	1	4.7	133	23	10	43.4
105	42	3	7.1	134	21	10	47.6
106	24	5	20.8	135	23	10	43.4
107	42	6	14.2	136	23	6	26.0
108	25	11	44.0	137	22	6	27.2
109	44	16	36.3	139	24	9	37.5
111	21	6	28.5	140	24	11	45.8
112	43	13	30.2	143	22	9	40.9
114	24	11	45.8	144	21	7	33.3
116	42	2	2.3	146	24	10	41.6
117	24	8	33.3	147	24	5	20.8
120	40	16	40.0	149	24	10	41.6
125	37	15	40.5	154	24	5	20.8
129	23	2	8.6				
20-F_3- 103	40	8	20.0	20-F_3- 120	25	3	12.0
104	24	8	33.3	123	20	7	35.0
105	24	6	25.0	125	22	5	22.7
107	24	2	8.3	127	22	5	22.7
108	24	9	37.5	129	23	2	8.6
109	41	6	14.6	133	23	7	30.4
110	39	16	41.0	135	22	3	13.5
111	25	12	48.0	140	21	10	47.6
114	39	15	38.4	142	20	9	45.0
116	25	5	20.0	145	21	5	23.8
117	41	15	36.5	146	23	7	30.4
118	24	7	29.1	148	20	4	20.0
119	39	9	23.0				

Susceptible F_3 families

19-F_3- 101	42	22	52.3	19-F_3- 123	42	21	50.0
113	24	16	66.6	124	24	24	100.0
115	24	12	50.0	126	22	11	50.0
119	24	13	54.1	138	25	13	52.0
121	24	12	50.0	141	24	13	54.1
122	25	25	100.0				
20-F_3- 101	19	17	89.4	20-F_3- 138	20	10	50.0
113	22	22	100.0	139	22	13	59.0
124	42	40	95.2	141	22	11	50.0
130	20	10	50.0				

TABLE 5. *Summary of Results with the F_3 Progenies of Hybrids 19, 20, Hull-less × Early Gothland, Inoculated with Ustilago levis*

	Total No.	Resistant	Segregating	Susceptible
F_2 plants not inoculated:				
Hybrid 19	24	3	9	12
Hybrid 20	22	1	8	13
	46	4	17	25
F_2 plants inoculated with *U. levis:*				
Hybrid 19	49	7	31	11
Hybrid 20	43	11	25	7
	92	18	56	18

From the group of second generation plants which had been inoculated with covered smut, 92 F_3 progenies were grown, the data being recorded in table 4 and summarized in table 5. There were 18 families containing 492 plants which were classified as resistant; 7 of these belonged to Hybrid 19 and 11 to Hybrid 20. Not a single infected plant was observed in any of these 18 progenies.

There were 56 progenies classified as segregating, the percentage of infection varying from 2.3 to 48 percent. Of these progenies, 31 belonged to Hybrid 19 and 25 to Hybrid 20. The 31 progenies of Hybrid 19 included 884 plants, of which 258 (29.1 percent) were smutted. The 25 progenies of Hybrid 20 included 671 plants, of which 185 (27.5 percent) were infected. Thus there was no important difference in the behavior of the progenies belonging to the two hybrids. Altogether, the 56 progenies included 1555 plants, of which 443 (28.4 percent) were infected.

There were 18 progenies which were classified as susceptible, since the amount of infection was greater than 50 percent. In 3 of these, all of the plants were infected, but in 12 others the percentage varied between 50 and 60 percent; the remaining 3 gave 66.6, 89.4, and 95.2 percent respectively. It is quite possible that the 12 families in which the amount of infection was little more than 50 percent really belonged in the segregating group.

The most interesting feature with reference to these results is the extreme preponderance of segregating and susceptible progenies. There were relatively few families which contained no smutted individuals. It might be expected that the second generation plants inoculated with the covered smut would not survive if they were susceptible, and thus no completely susceptible progenies would be found in the next generation. Actually, we find not only a high percentage of infection of the second generation plants, but also a corresponding high percentage of susceptibility in the third generation. However, only 5 of the third generation progenies which had survived inoculation in the second generation gave approximately 100 percent of smutted plants. Many of the remaining ones showed barely more than 50 percent infection.

Data Obtained with the F_4 Generation

As recorded in table 5, there were 22 F_3 progenies resistant to the covered smut, 4 of which descended from uninoculated F_2 plants and 18 descended from F_2 plants which had been inoculated with covered smut. Fourth generation descendants were grown from 15 of these 22 progenies, all 4 of the first group being represented and 11 of the second. With one exception, seed was taken from 6 different third generation plants of each progeny and inoculated with the covered smut. Altogether, 88 fourth generation progenies of these 15 resistant third generation families were grown, and the results obtained are recorded in the form of a summary in table 6.

TABLE 6. *Summary of Results with F_4 Progenies of Hybrids 19, 20, Early Gothland × Hull-less, Descended from F_3 Progenies Resistant to Ustilago levis.*

	F_4 generation, Inoculated with Ustilago levis			
	Number of Progenies	Number of Progenies Infected	Number of Plants	Number of Plants Infected
F_2 not inoculated; F_3 inoculated with *Ustilago levis:*				
Hybrid 19-F_3- 3	6	1	143	1
18	6	2	142	3
19	6	5	147	6
Hybrid 20-F_3-13	6	2	144	2
	24	10	576	12
F_2 and F_3 inoculated with *Ustilago levis:*				
Hybrid 19-F_3-110	4	3	85	4
118	6	2	138	2
127	6	2	143	2
128	6	2	141	2
131	6	1	141	1
Hybrid 20-F_3-102	6	2	145	2
106	6	0	139	0
112	6	0	143	0
115	6	0	143	0
121	6	1	144	1
122	6	3	143	3
	64	16	1505	17

One of the noteworthy features of the results is the fact that 26 of these fourth generation progenies contained one or more smutted plants. The total number of plants grown in all the progenies was 2081 and 29 (1.3 per-cent) were infected.

Some infected plants were found in the progenies descended from each of the 4 second generation plants which had not been inoculated. Further, among the fourth generation descendants of the F_2 plants which had been inoculated with covered smut, smutted plants were found in all except 3.

These 3 F_3 progenies were represented by 18 fourth generation progenies descended from entirely resistant families in the third generation and these in turn came from inoculated but resistant F_2 plants.

The general results obtained with the hybrids of Early Gothland × Hull-less are interesting in view of the comparatively high percentage of second generation plants which were infected by the covered smut. We further find in the third generation a comparatively small number of pure resistant progenies. There was a great excess of progenies which gave 2.3–48.7 percent infection; there was also a considerable number which gave more than 50 percent infection. Finally, we have the further interesting situation in which infected plants show up in the fourth generation progenies, although both parental third and second generations had been resistant.

Hybrids of Monarch and Hull-less

The F_1 plants of four crosses between these two varieties were grown in 1926 and the first series of second generation plants in 1927. The F_2 plants were divided into three sets, one of which was inoculated with the covered smut, another set with the loose smut, and a third set was grown to maturity without inoculation. Additional plants from each of the four hybrids were grown in 1928 in the field. Some of these were inoculated with the covered smut and others with the loose. Brief reports on some of the results with these hybrids have been published (12, 13, 14, 16). The data for all the F_2 plants are recorded in table 1.

Data Obtained with the F_2 Generation

Results with Ustilago levis. In 1927, 25 plants of Hybrid 24 were inoculated with the covered smut and 22 (88 percent) were infected; 27 plants of Hybrid 25 were inoculated and all of them were infected; 23 plants of Hybrid 26 were inoculated and 22 (95.6 percent) were infected; 25 plants of Hybrid 27 were inoculated and 24 (96 percent) were infected. A few additional plants of each of the hybrids were inoculated with the covered smut in 1928 and in every case 100 percent infection was secured. Altogether, 148 F_2 plants belonging to the four hybrids were inoculated and 143 (96.6 percent) were infected. Thus the second generation hybrids exhibited susceptibility to the covered smut corresponding to that of the two parental varieties, Monarch and Hull-less.

Results with Ustilago Avenae. A great many second generation plants were inoculated with the loose smut. In 1927, 49 F_2 plants of Hybrid 24 were inoculated and 12 (24.4 percent) were infected; 50 plants of Hybrid 25 were inoculated and 11 (22 percent) were infected; 48 plants of Hybrid 26 were inoculated and 7 (14.5 percent) were infected; 49 plants of Hybrid 27 were inoculated and 11 (22.4 percent) were infected. The percentage of infection in the different hybrids varied from 14.5 to 24.4 percent. In 1928 approximately the same number of plants of each of these hybrids were

inoculated with the loose smut and grown to maturity in the field; in every case higher percentages of infection were obtained than in the previous season. In 1928 the lowest percentage of infection was 38.5 percent with Hybrid 27, and the highest 43.6 percent with Hybrid 25. Combining all of the results secured in the two years, 415 F_2 plants belonging to the four hybrids were inoculated and 130 (31.3 percent) were infected.

The results indicate that resistance to loose smut is dominant in the second generation of these hybrids. It is noteworthy, however, that the percentage of infected plants is higher than that usually obtained in my experiments with various oat hybrids. Generally, the second generation has given percentages of infection slightly below 25 percent. It may also be noted that a much larger number of infected plants was obtained in 1928 as compared with 1927.

The results with the second generation of these hybrids inoculated with the loose smut, however, correspond somewhat closely to those just described for Hybrids 19 and 20, inoculated with the covered smut. In both sets of hybrids there has been a comparatively high percentage of infection among the second generation plants.

Data Obtained with the F_3 Generation

Investigations with these hybrids were continued with a large number of third generation progenies. There were available for the study of smut resistance in this generation three classes of families: (1) the 5 F_2 plants which had been inoculated with covered smut and failed to show infection; (2) the plants which had been inoculated with loose smut and survived; and (3) the uninoculated F_2 plants.

Results with Ustilago levis. Third generation progenies of 4 of the F_2 plants which survived the inoculation with the covered smut were reinoculated with this same smut. These 4 progenies included 51 plants of which 47 (92.1 percent) were infected (table 7). These results indicate that the F_2 plants are merely chance escapes.

There were grown 12 progenies of each of the 4 hybrids descended from the uninoculated F_2 plants, the data being recorded in table 7. These 48 progenies included 854 plants, of which 832 (97.4 percent) were infected. There were also grown 12 progenies of each of the four hybrids descended from F_2 plants which had been inoculated with loose smut; these contained 844 plants, of which 826 (97.8 percent) were infected. Thus all of these 96 progenies belonging to these four hybrids, one half of which descended from uninoculated F_2 plants and the other half from F_2 plants inoculated with loose smut, have shown practically complete susceptibility to the covered smut. Certainly they have manifested a susceptibility entirely comparable to that of the parental varieties, Monarch and Hull-less.

Results with Ustilago Avenae. In view of the behavior of the second generation plants, more extensive data have been obtained with the loose

TABLE 7. *Results with the F_3 Progenies of Hybrids 24 to 27, between Monarch and Hull-less, Inoculated with Ustilago levis*

Hybrid and Progeny Number	Total No. Progenies	Total No. Plants	No. Inf.	Percent Inf.
Descended from uninoculated F_2 plants				
Hybrid 24-F_3-1, 2, 3, 4, 6, 8, 9, 10, 12, 14, 15, 20	12	221	213	96.3
Hybrid 25-F_3-1, 3, 8, 10, 13, 14, 15, 18, 21, 22, 23, 25	12	222	218	98.1
Hybrid 26-F_3-1, 2, 6, 7, 8, 9, 10, 15, 16, 17, 19, 21	12	209	204	97.6
Hybrid 27-F_3-1, 2, 3, 4, 6, 7, 9, 10, 11, 14, 18, 19	12	202	197	97.5
	48	854	832	97.4
Descended from F_2 plants inoculated with Ustilago Avenae				
Hybrid 24-F_3-101, 102, 103, 105, 106, 111, 112, 116, 119, 126, 127, 131	12	220	216	98.1
Hybrid 25-F_3-102, 106, 109, 110, 112, 114, 122, 123, 125, 127, 129, 135	12	217	211	97.2
Hybrid 26-F_3-106, 114, 116, 120, 121, 125, 126, 127, 137, 138, 139, 141	12	217	211	97.2
Hybrid 27-F_3-101, 105, 106, 107, 111, 115, 116, 119, 120, 121, 128, 129	12	190	188	98.9
	48	844	826	97.8
Descended from F_2 plants inoculated with Ustilago levis				
Hybrid 24-F_3-52, 53	2	24	23	95.8
Hybrid 26-F_3-51	1	14	13	92.8
Hybrid 27-F_3-51	1	13	11	84.6
	4	51	47	92.1

smut on various third generation progenies. The data on these progenies are recorded in tables 8 and 9 and summarized in table 10. Results were obtained with two groups of third generation progenies: (1) those descended from uninoculated F_2 plants and (2) those descended from F_2 plants which had been inoculated with loose smut. In the tables, the families are grouped on the basis of their behavior—whether resistant, segregating, or susceptible.

1. Results obtained with F_3 progenies descended from uninoculated F_2 plants. The data for these progenies are recorded in table 8. There were 90 progenies grown, nearly equally distributed among the four hybrids. Summarized in table 10, these 90 third generation progenies were classified into three groups. There were 9 resistant progenies, 3 belonging to each of the Hybrids 24, 25, and 26; in none of these were any infected plants observed. There were 51 progenies classified as segregating, the percentage of infection varying from 4 to 48.2 percent. Finally, there were 30 progenies in which more than half of the inoculated plants were infected; only one of these progenies, however, gave 100 percent infection, 33 plants being grown.

TABLE 8. *Results with the F_3 Progenies of Hybrids 24 to 27, between Monarch and Hull-less, Descended from Uninoculated F_2 Plants. All Progenies Inoculated with Ustilago Avenae*

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
Resistant F_3 families							
24-F_3- 16	25	0	0	27-F_3- 5	18	0	0
21	23	0	0	12	25	0	0
23	22	0	0	15	25	0	0
25-F_3- 4	22	0	0				
11	25	0	0				
24	23	0	0				
Segregating F_3 families							
24-F_3- 3	36	15	41.6	26-F_3- 1	29	14	48.2
4	33	7	21.2	2	24	1	4.1
5	24	6	25.0	4	25	6	24.0
7	25	1	4.0	5	21	2	9.5
11	24	5	20.8	6	32	15	46.8
13	23	7	30.4	8	23	1	4.3
15	28	11	39.2	9	33	11	33.3
17	24	9	37.5	10	27	11	40.7
18	25	12	48.0	11	21	3	14.2
19	25	7	28.0	17	22	1	4.5
20	29	8	27.5	18	24	11	45.8
22	25	10	40.0				
24	23	2	8.6				
25-F_3- 1	28	13	46.4	27-F_3- 2	29	8	27.5
3	25	3	12.0	3	32	15	46.8
7	23	6	26.0	7	35	16	45.7
9	23	6	26.0	10	34	15	44.1
10	32	12	37.5	11	28	5	17.8
12	23	7	30.4	13	21	2	9.5
13	30	12	40.0	16	23	4	17.3
14	28	10	35.7	17	24	1	4.1
15	23	1	4.3	18	38	6	15.7
17	22	1	4.5	20	24	9	37.5
19	21	5	23.8	22	23	2	8.6
20	23	9	39.1	23	24	7	29.1
21	32	12	37.5				
23	31	14	45.1				
25	39	18	46.1				
Susceptible F_3 families							
24-F_3- 1	35	24	68.5	26-F_3- 3	20	11	55.0
2	38	34	89.4	7	42	27	64.2
6	39	37	94.8	13	22	17	77.2
8	28	24	85.7	15	16	15	93.7
9	36	23	63.8	16	38	23	60.5
10	33	33	100.0	19	39	25	64.1
12	41	25	60.9	20	24	16	66.6
14	39	30	76.9	21	43	33	76.7
25-F_3- 2	23	15	65.2	27-F_3- 1	39	32	82.0
5	23	12	52.1	4	37	26	70.2
8	35	22	62.8	6	42	32	76.1
16	23	17	73.9	8	23	16	69.5
18	33	18	54.5	9	43	22	51.1
22	39	22	56.4	14	39	38	97.4
				19	19	17	89.4
				21	24	14	58.3

TABLE 9. *Results with the F_3 Progenies of Hybrids 24 to 27, between Monarch and Hull-less, Descended from F_2 Plants which had been Inoculated with Ustilago Avenae. All Progenies Inoculated with Ustilago Avenae*

Resistant F_3 families

Hybrid and Progeny Number	Total No. Progenies	Total No. Plants	No. Inf.	Percent Inf.
Hybrid 24-F_3- 106, 109, 113, 115, 128, 134, 136, 141, 145, 150, 152, 158, 161, 162, 163	15	353	0	0
Hybrid 25-F_3- 105, 107, 110, 111, 123, 126, 129, 134, 137, 147, 148, 155, 156, 163, 165	15	389	0	0
Hybrid 26-F_3- 101, 107, 114, 117, 118, 128, 134, 140, 146, 159, 165, 167, 168, 169	14	307	0	0
Hybrid 27-F_3- 103, 110, 114, 122, 124, 127, 145, 151, 157, 162	10	231	0	0
	54	1280	0	0

Segregating F_3 families

Hybrid No.	No. Plants	No. Inf.	Percent Inf.	Hybrid No.	No. Plants	No. Inf.	Percent Inf.
24-F_3- 101	37	10	27.0	25-F_3- 101	23	2	13.0
102	32	2	6.2	102	42	9	21.4
103	30	9	30.0	103	22	6	27.2
104	25	5	20.0	106	35	7	20.0
105	35	7	20.0	108	24	4	16.6
108	24	8	33.3	113	22	2	9.0
110	23	11	47.8	114	40	10	25.0
111	31	4	12.9	116	24	8	33.3
112	30	3	10.0	117	23	4	17.3
114	24	5	20.8	118	22	2	9.0
116	35	9	25.7	119	22	7	31.8
117	20	2	10.0	120	22	6	27.2
118	25	9	36.0	121	22	1	4.5
119	38	11	28.9	124	22	8	36.3
121	24	7	29.1	125	39	11	28.2
122	22	8	36.3	127	36	14	38.8
123	23	3	13.0	128	25	5	20.0
125	22	4	18.1	131	25	10	40.0
126	41	10	24.3	132	22	7	31.8
127	39	3	7.6	133	23	9	39.1
130	24	7	29.1	135	29	8	27.5
131	38	9	23.6	136	24	7	29.1
132	24	3	12.5	138	24	11	45.8
135	23	4	17.3	139	21	1	4.7
137	20	2	10.0	140	22	1	4.5
138	22	4	18.1	141	22	5	22.7
139	24	5	20.8	142	25	2	8.0
140	25	1	4.0	143	24	3	12.5
142	22	5	22.7	144	22	8	36.3
143	24	1	4.1	145	23	7	30.4
144	24	1	4.1	146	24	1	4.1
146	23	10	43.4	149	23	1	4.3
148	23	9	39.1	150	22	10	45.4
149	22	4	18.1	151	25	6	24.0
151	22	3	13.5	152	23	2	8.6
153	25	7	28.0	154	20	4	20.0
154	22	8	36.3	158	25	4	16.0
155	25	1	4.0	159	22	1	4.5
156	22	4	18.1	160	25	2	8.0
157	25	10	40.0	162	20	8	40.0
159	24	5	20.8	166	22	3	13.5
160	22	8	36.3	168	24	1	4.1
				169	25	1	4.0

TABLE 9.—*Continued*

Hybrid No.	No. Plants	No. Inf.	Percent Inf.
26-F_3- 102	24	4	16.6
103	23	7	30.4
104	24	7	29.1
105	24	8	33.3
106	19	1	5.2
108	17	1	5.8
109	22	1	4.5
110	23	1	4.3
111	22	3	13.5
112	20	1	5.0
113	24	8	33.3
116	28	11	39.2
119	23	1	4.3
120	31	7	22.5
121	32	14	43.7
122	24	6	25.0
123	24	2	8.3
124	20	4	20.0
125	21	1	4.7
126	25	11	44.0
129	23	8	34.7
130	23	7	30.4
131	25	7	28.0
132	21	2	9.5
133	23	6	26.0
135	24	6	25.0
136	21	3	14.2
137	31	7	22.5
138	37	18	48.6
139	24	6	25.0
141	24	2	8.3
142	23	4	17.3
143	25	4	16.0
145	25	6	24.0
147	23	1	4.3
148	24	2	8.3
149	23	6	26.0
150	24	2	8.3
151	25	11	44.0
152	24	3	12.5
153	22	8	36.3
154	25	3	12.0
155	25	2	8.0
156	24	5	20.8
157	22	3	13.5
158	23	3	13.0
160	21	2	9.5
162	19	1	5.2
163	20	5	25.0
164	23	7	30.4
166	23	6	26.0
27-F_3- 101	41	9	21.9
102	21	4	19.0
104	22	4	18.1
105	36	16	44.4
106	35	12	34.2
107	32	14	43.7
108	24	6	25.0
113	22	9	40.9
116	24	1	4.1
118	23	4	17.3
120	32	14	43.7
121	35	14	40.0
123	22	3	13.5
125	22	6	27.2
126	25	11	44.0
128	24	6	25.0
130	25	3	12.0
131	21	5	23.8
132	22	1	4.5
134	23	6	26.0
135	24	10	41.6
136	23	10	43.4
137	24	10	41.6
138	21	5	23.8
141	23	6	26.0
142	24	6	25.0
143	23	5	21.7
144	24	1	4.1
146	24	6	25.0
147	24	4	16.6
148	21	1	4.7
150	24	1	4.1
153	21	4	19.0
154	25	3	12.0
155	22	9	40.9
156	22	7	31.8
158	22	9	40.9
159	22	9	40.9
161	23	1	4.3
163	22	8	36.3
164	24	4	16.6
165	24	8	33.3
166	25	10	40.0
167	20	9	45.0
168	24	3	12.5

Susceptible F_3 families

24-F_3- 107	25	16	64.0	26-F_3- 115	24	12	50.0
120	24	13	54.1	127	42	25	59.0
124	23	19	82.6				
129	21	11	52.3				
133	24	12	50.0				
147	24	12	50.0				
25-F_3- 104	23	20	86.9	27-F_3- 109	22	12	54.5
109	33	20	60.6	111	43	29	67.4
112	42	24	57.1	112	25	19	76.0
115	25	21	84.0	115	28	14	50.0
122	42	25	59.5	119	33	23	69.6
130	22	19	86.3	129	35	21	60.0
153	22	18	81.8	139	22	14	63.6
161	25	14	56.0	140	24	12	50.0
164	22	12	54.5	152	25	14	56.0

TABLE 10. *Summary of Results with the F_3 Progenies of Hybrids 24 to 27, Monarch and Hull-less, Inoculated with Ustilago Avenae*

	Total No.	Resistant	Segregating	Susceptible
F_2 plants not inoculated:				
Hybrid 24	24	3	13	8
Hybrid 25	24	3	15	6
Hybrid 26	19	0	11	8
Hybrid 27	23	3	12	8
	90	9	51	30
F_2 plants inoculated with *U. Avenae:*				
Hybrid 24	63	15	42	6
Hybrid 25	67	15	43	9
Hybrid 26	67	14	51	2
Hybrid 27	64	10	45	9
	261	54	181	26

The percentage of infection in three additional progenies ranged between 90 and 100 percent. There were, however, 18 of the progenies which gave percentages of infection varying between 50 and 75 percent.

Two distinct features stand out in these results with the third generation. One is the comparatively small number of pure resistant third generation progenies, only 9 out of a total of 90 being in this group. A second feature is the comparatively low percentages of infection obtained with the so-called susceptible families. Relatively few of them gave infections at all approaching 100 percent.

2. Progenies descended from F_2 plants inoculated with loose smut. The data are recorded in detail in table 9 and summarized in table 10. More than 60 progenies of each of the four hybrids belonging to this group were inoculated, altogether 261 being grown. There were 54 of these progenies classified as resistant, entirely negative results having been secured; these included 1280 plants. There were 181 progenies classified as segregating, the percentage of infection varying from 4 to 48.6 percent; these were somewhat equally distributed among the four hybrids. There were 42 segregating progenies of Hybrid 24; these included 1105 plants, of which 241 (21.8 percent) were infected. Hybrid 25 contained 43 segregating progenies, which included 1071 plants, 229 (21.3 percent) being infected. Hybrid 26 included 51 segregating progenies containing 1209 plants, of which 255 (21 percent) were infected. Finally, Hybrid 27 included 45 segregating progenies, which contained 1106 plants, 297 (26.8 percent) being infected. Altogether, the 181 progenies included 4491 plants, of which 1022 (22.7 percent) were infected.

The remaining 26 progenies were classified as susceptible, since more than 50 percent of smutted plants were obtained. An examination of the details of table 9, however, reveals the interesting fact that 15 of these

progenies gave between 50 and 60 percent infection. Only 5 gave percentages of infection above 80 percent and none were found in which all the plants were smutted.

The results indicate a low proportion of completely resistant third generation progenies, as compared with the progenies in the segregating group. While 26 progenies were classified as susceptible, it is quite possible that many of them actually belonged in the segregating group, since the amount of infection was barely more than 50 percent.

Data Obtained with the F_4 Generation

A few experiments have been carried out with the fourth generation of these hybrids. As recorded in tables 8, 9, and 10, there were 63 third generation progenies which proved to be entirely resistant to the loose smut, 9 of these having descended from uninoculated second generation plants and the remaining 54 from F_2 plants which had been inoculated with the loose smut. Descendants of many of these third generation progenies have been grown in the fourth generation and, for the sake of clearness, they are considered on the basis of the treatment of the F_2 plants.

1. The F_2 plant not inoculated. There were 9 resistant third generation progenies descended from uninoculated F_2 plants, and fourth generation progenies from 8 of these were grown. In each case seed was taken from 6 different F_3 plants of each progeny and inoculated with the loose smut.

The data are recorded in table 11. This table shows the number of progenies grown from each F_2 plant, and the number of progenies which contained one or more infected individuals. The table also shows the total number of individuals grown and the number infected.

Altogether there were 48 F_4 progenies grown from the 8 third generation progenies, and 24 of these contained one or more smutted plants. The total number of individual plants grown was 1063, of which 52 (4.8 percent) were smutted. The highest percentage of infection obtained in any fourth generation progeny was 20.8 percent.

Of the 8 second generation plants represented by descendants in the fourth generation, only 2 showed complete freedom from smut, having a consistent record of smut resistance in the third and fourth generations.

2. The F_2 plant inoculated with loose smut. As recorded in tables 9 and 10, there were 54 third generation progenies descended from F_2 plants inoculated with the loose smut which showed complete resistance. The fourth generation descendants of 23 of these progenies were grown and in nearly every case there were 6 families from each separate F_3 progeny. Altogether, there were 136 fourth generation progenies and 62 of these contained one or more smutted plants. The total number of individuals grown was 3069, of which 150 (4.8 percent) were infected. The percentage of infection was usually quite low; the highest obtained was 39.1 percent. It is interesting to note that a larger number of infected individuals occurred in the progenies of Hybrids 24 and 27.

TABLE II. *Summary of Results with F_4 Progenies of Hybrids 24–27, Monarch and Hull-less Descended from F_3 Progenies Resistant to Ustilago Avenae*

	F_4 generation, Inoculated with Ustilago Avenae			
	Number of Progenies	Number of Progenies Infected	Number of Plants	Number of Plants Infected
F_2 not inoculated; F_3 inoculated with *Ustilago Avenae:*				
Hybrid 24-F_3- 16	6	3	136	4
21	6	5	148	8
23	6	0	126	0
Hybrid 25-F_3- 4	6	2	137	6
11	6	4	141	13
24	6	5	136	13
Hybrid 27-F_3- 5	6	0	126	0
12	6	5	113	8
	48	24	1063	52
F_2 and F_3 inoculated with *Ustilago Avenae:*				
Hybrid 24-F_3- 106	6	5	140	12
109	6	3	129	13
113	6	0	141	0
115	6	5	143	13
Hybrid 25-F_3- 105	6	2	141	2
107	6	2	139	2
110	6	0	138	0
111	6	4	136	5
123	6	1	134	1
Hybrid 26-F_3- 101	6	1	137	1
107	6	6	134	7
114	6	0	141	0
117	6	4	131	5
118	6	0	144	0
128	6	3	126	3
134	4	1	90	2
140	6	4	138	5
Hybrid 27-F_3- 103	6	6	144	40
110	6	6	136	18
114	6	1	113	1
122	6	4	139	15
124	6	2	135	3
127	6	2	120	2
	136	62	3069	150

Only 4 of the 23 F_2 plants showed complete resistance in both the third and fourth generations. One of these was found in Hybrid 24, one in Hybrid 25, and two in Hybrid 26, Hybrid 27 not being represented.

DISCUSSION

During the past ten years a number of contributions on the inheritance of smut resistance in oats have been made. Wakabayashi (26), in 1921,

published data on the behavior of a cross between Red Rustproof and Black Tartarian towards covered smut. In his experiments, Red Rustproof was a resistant variety, while Black Tartarian produced from 25 to 40 percent infection. No smut was observed in either the F_1 or F_2 generation. In the third generation, however, infected plants were found in 12 progenies, the percentage varying from .01 to 15 percent; 95 additional progenies were entirely resistant. The most susceptible F_3 progeny contained less than half as much smut as the susceptible parent. Wakabayashi concluded that resistance to the covered smut was dominant, and was inclined to explain his data on the basis of three independent factors for resistance in the Red Rustproof variety.

Barney (2), in 1924, published his results on the resistance to loose smut of certain oat crosses. He grew 158 F_2 plants of a cross between two very resistant varieties—Fulghum and Black Mesdag—and only 2 were infected. In a cross between the very susceptible Swedish Select variety and the resistant Burt, he obtained 144 normal F_2 plants and 10 infected. In a third cross between the two susceptible varieties Turkish Rustproof and Gold Rain, 259 normal F_2 plants were observed, and 56 smutted. Barney explained his results on the supposition that, in the first cross, inheritance of smut resistance is dependent upon three independent factors, in the second cross, upon two factors and, in the third, upon one.

Barney also studied the behavior of 70 third generation families of a cross between Sixty-Day and Black Mesdag. Of these families 22 were found to be resistant, 32 gave percentages of infection of 20 percent or less, and the remaining 16 families gave more than 20 percent infection. No data on the comparative behavior of the parents are given. Barney interpreted his results with these third generation progenies on the basis of one factor difference between the parents. Descendants of some of these progenies were grown in later generations and definitely resistant and susceptible strains were obtained.

In 1925, Reed and Stanton (21) published their results on the behavior of 92 third generation families of a cross between Fulghum and Swedish Select. These were inoculated with the loose smut, to which Swedish Select is very susceptible, and Fulghum highly resistant. A wide range of susceptibility of the families was noted; 25 families showed a resistance comparable to that of the Fulghum parent, giving less than 5 percent infection, and 12 of these gave entirely negative results; 33 families gave 5 to 20 percent infection; 22 families 20 to 40 percent infection; and 12 families infections greater than 40 percent. It was also found that fourth generation selections from susceptible F_3 families proved to be highly susceptible, and similar selections from resistant F_3 families in general proved to be resistant. A few selections were also grown in the fifth generation. The interesting fact was determined that the fourth and fifth generations appeared to behave in a similar fashion to both loose and covered smut.

In 1925, I described my experiments with a hybrid of Hull-less × Black Mesdag, inoculated with loose smut (10). Black Mesdag is completely resistant to the loose smut, while Hull-less has shown a high degree of susceptibility. In the F_2 generation, 82 plants were inoculated and 21 (25.6 percent) were infected. Two groups of third generation progenies were grown, one descended from the resistant F_2 plants, and the other descended from uninoculated F_2 plants. Of the first group there were 42 F_3 progenies grown, of which 12 were resistant, 30 segregating, and none susceptible. There were 58 progenies of the second group grown and 18 were resistant, 28 segregating, and 12 susceptible. A few progenies were also grown in the F_4 generation. All of the facts obtained were in close harmony with the view that resistance to the loose smut in this hybrid was dominant and segregation occurred on the basis of a three to one ratio.

Gaines (3, 4), in 1925, referred briefly to some data on crosses between Black Tartarian, Abundance, and Large Hull-less, varieties highly susceptible to covered smut, combined with Red Rustproof. Resistance to the smut was found to be dominant; only 5 of the 612 F_3 progenies grown were as susceptible as the susceptible parent. In the cross between Black Tartarian and Red Rustproof, only 10 percent of the F_3 families produced any smut, and the most severely infected produced only 15 percent, as compared with 34 percent for the Black Tartarian parent. In the cross with Abundance and Red Rustproof, 9 families out of a total of 345 produced more than 5 percent of smut, 286 families being entirely free. In the cross between Large Hull-less and Red Rustproof, 46 families out of a total of 149 showed some infection. The most susceptible progeny produced only 51 percent of smut, as compared with 86 percent with the Large Hull-less parent.

In 1928, I published very extensive data on a number of different oat hybrids (13). Investigations were carried out with four additional crosses between Hull-less and Black Mesdag, both second and third generations, inoculated with loose and covered smut, being grown. There were 465 F_2 plants, belonging to the four crosses, inoculated with loose smut and 107 (23 percent) were infected. There were 196 F_2 plants of these same hybrids inoculated with covered smut and 40 (20.4 percent) were infected. Altogether, 578 third generation families belonging to the four hybrids were inoculated with each smut and of these 541 gave a similar reaction. If a progeny was resistant to one smut, it was resistant to the other; if it was susceptible to one, it was susceptible to the other. The remaining 49 progenies, however, showed a dissimilar behavior. All of the data suggest a single factor difference for resistance in these two varieties, and a very close parallelism in the reaction of the hybrids to loose and covered smut.

Second generation plants of a cross between Silvermine and Black Mesdag were also grown. There were 95 F_2 plants inoculated with the loose smut and 17.8 percent of these were infected. At the same time, 89 F_2 plants

inoculated with covered smut gave 16.8 percent infection. In these hybrids resistance to both smuts again appeared to be dominant.

Results were also obtained with hybrids between certain susceptible varieties. There were two such crosses, one between Canadian and Victor, and the other between Silvermine and Hull-less. All of these varieties are susceptible to both loose and covered smut. There were 91 F_2 plants of Canadian × Victor and 53 F_2 plants of Silvermine × Hull-less inoculated with the loose smut, and every plant was infected. There were 42 F_2 plants of the hybrid Canadian × Victor inoculated with the covered smut, and all were infected. Thus the second generation plants of these hybrids are completely susceptible, like the parental varieties.

In this same paper were published the first data obtained on the F_2 plants of Hybrids 19 and 20, Early Gothland × Hull-less, and Hybrids 24–27 between Monarch and Hull-less.

Hayes, Griffee, Stevenson, and Lunden (6), in 1928, presented some data on a cross between an oat hybrid selection, combined with Black Mesdag. A mixture of the spores of loose and covered smut was used for inoculation, and 378 F_3 progenies were grown. In recording their data, the percentage of infected plants was multiplied by the average percentage of infected panicles. The significance of this procedure is not explained. The susceptible parent gave percentages of infection varying from 3.2 to 17.1 percent in the different rows. Of the F_3 progenies, 47 gave no infection, 86 were classified as susceptible, the percentage of infection on an average being about 4.4 percent, and the remaining families gave only small traces of smut. They suggest as an explanation of their results the occurrence of two distinct factors, one for immunity and the other for resistance, both carried in the Black Mesdag parent.

In 1928, Garber, Giddings and Hoover (5) published their data on a cross between Gopher and Black Mesdag, using a mixture of spores of loose and covered smut. The Gopher parent, in 11 different plots, gave from none to 37.5 percent infection, and Black Mesdag gave negative results. They grew 100 F_3 progenies of the hybrid, and 21 gave no infection, 74 gave an infection from 2.5 to 42.5 percent, and 4 a percentage of infection greater than 50. They also followed some of these families in the F_4 and later generations, and lay special emphasis upon the point that some of the progenies are more susceptible than the Gopher parent. They interpreted their results on the basis that the inheritance of resistance is controlled by a single main factor difference but, in addition, that there is at least one supplementary factor brought in by the Black Mesdag parent, which accounts for the so-called transgressive segregation for susceptibility.

Von Rosenstiel (22), in 1929, published his paper on the inheritance of resistance to loose smut of four crosses between Black Mesdag (Mesdago) and four susceptible varieties. He also conducted extensive experiments on the different methods of infection, including the study of those which I have

worked out, and he came to the conclusion that my methods were particularly effective for determining the resistance of varieties and hybrids. Four susceptible varieties—Carsten III, Dippes Überwinder, Peragis, and Stamm 01108, which gave from 93.7 to 100 percent infection, were crossed with Black Mesdag. His results were as follows: 35 F_3 families of Carsten III × Black Mesdag were grown, and 11 were resistant, 16 segregating, and 8 susceptible; 89 F_3 families of Dippes Überwinder × Black Mesdag were grown, and 29 were resistant, 40 segregating, and 20 susceptible; 20 F_3 families of Peragis × Black Mesdag were grown, and 4 were resistant, 11 segregating, and 5 susceptible; 192 F_3 families of Stamm 01108 × Black Mesdag were grown, and 68 were resistant, 75 segregating, and 49 susceptible.

In 1931, Nicolaisen (7) published his results on the methods of infecting oats by loose smut, with a view to determining the resistance of varieties, and also the inheritance of smut resistance, and has found that the methods which I have developed are very effective. The inheritance of resistance in three different crosses was studied. He grew 189 F_3 progenies of v. Lochows Gelbhafer × Black Mesdag (Mesdago); 67 of these gave no infection, 93 gave infections varying from 1 to 15 percent, 26 from 15.1 to 50 percent, and 3 more than 50 percent. In the cross v. Lochows Gelbhafer × Red Rustproof, out of 114 progenies, 63 were resistant, 38 gave infections from 1 to 15 percent, 9 from 15.1 to 50 percent, and 4 above 50 percent. The hybrid Red Rustproof × Stamm 01108 contained 107 F_3 progenies, 42 being resistant, 28 giving infections varying from 1 to 15 percent, 32 progenies from 15.1 to 50 percent, and 5 others more than 50 percent. The variety v. Lochows Gelbhafer in 1928 and 1929 gave slightly more than 10 percent infection, Stamm 01108 gave infections from 97.9 to 99.3 percent, and Black Mesdag and Red Rustproof were entirely negative. Nicolaisen explains the behavior of his progenies for the first two crosses on the basis of a three-factor difference and the behavior of the third cross on the basis of a difference of two factors.

Welsh (27) has recorded some limited data on the F_3 progenies of hybrids between Victory, a variety susceptible to both loose and covered smuts, and a resistant selection from a cross involving Minota, White Russian, and Black Mesdag. He found that 118 F_3 progenies were resistant to loose smut and 183 contained infected individuals. Of these same progenies, 126 were resistant to covered smut and 175 contained smutted plants. There were 87 progenies resistant to both, while the remaining 224 progenies contained plants infected by one or the other or both loose and covered smut. Welsh suggests some relationship between the infecting capacities of the two smuts on these hybrids. He also found that some of the third generation progenies gave higher percentages of infection than the susceptible Victory parent. To account for such results he concluded that at least two factors governed the smut reaction, resistance being dominant.

In 1931, I published (17) my results on the inheritance of smut resistance in three crosses between Early Gothland and Monarch. As repeatedly emphasized, these varieties differ in that Early Gothland is very susceptible to loose smut and resistant to the covered, while Monarch is exactly the reverse. Data were secured on the second, third, and fourth generations, separate sets of plants being inoculated with loose and covered smut. The evidence indicated that the factors for resistance to these two smuts are independently inherited in these hybrids. A progeny resistant, segregating, or susceptible to the loose smut might belong to any one of these classes in its behavior to the covered smut. It was possible, however, to combine in the same selection or strain resistance to both smuts.

The results with the inoculated second generation plants of these crosses indicated that resistance was dominant to both. There were 50 F_2 plants inoculated with the loose smut and 9 (18 percent) were infected, and 86 F_2 plants were inoculated with the covered smut, 9 (10.4 percent) being infected. The data for the third and fourth generations corresponded. While the data were not sufficiently extensive, yet all the facts seemed to fit the interpretation that segregation for resistance to each smut, independently of the other, in the second generation occurred in a ratio of three to one.

In a very recent paper (18) I have published data on a hybrid between Early Gothland and Victor, the latter variety being very susceptible to both smuts. Second and third generation plants were inoculated with loose and covered smut. Altogether, 67 F_2 plants were inoculated with the loose smut and all became infected. A very high percentage of infection was also secured with the few third generation progenies inoculated with this smut. Thus the hybrids were, like the two parental varieties, susceptible.

Much more extensive data were secured with the covered smut. In the F_2 generation, 147 plants were inoculated, of which 16 (10.8 percent) were infected; these F_2 plants were grown at three different times, the percentage of infection varying from 4 to 18.1 percent. A large number of third generation progenies inoculated with the covered smut were also grown. Some of these progenies descended from uninoculated F_2 plants; 52 progenies of this group were grown and 18 were entirely resistant, 27 segregating, and 7 susceptible. A larger number of F_3 progenies, descended from F_2 plants which had been inoculated with the covered smut, were grown. Altogether, there were 107 F_3 progenies of this group, 43 being resistant and 64 segregating.

The results show that resistance to covered smut is dominant, although the percentage of infected F_2 plants is too small to indicate clearly segregation on the basis of a three to one ratio. Further, in the third generation we find too many resistant progenies. However, it is interesting to note that if these progenies descended from uninoculated F_2 plants, we find pure resistant progenies, segregating progenies, and pure susceptible progenies, a result exactly according to expectation. Further, among the progenies descended

from F_2 plants inoculated with the covered smut, we find only the two groups of resistant and segregating, no susceptible progenies being observed, a result also according to expectation. In both groups, however, there was an excessive preponderance of resistant families.

The hybrids Early Gothland × Victor and Early Gothland × Hull-less offer exactly the same combination of varieties with regard to their behavior to the two smuts. Early Gothland is susceptible to loose smut and resistant to the covered, while Victor and Hull-less are quite susceptible to both smuts. It is, then, surprising that the results with the hybrids of these two sets of crosses should be so different. In Early Gothland × Victor we find a great preponderance of resistance in both the F_2 and F_3 generations. On the other hand, the hybrids between Early Gothland and Hull-less show a high degree of susceptibility, almost 50 percent of the second generation plants being infected. There is also a very high proportion of segregating or susceptible F_3 progenies. Finally, relatively few fourth generation progenies are pure resistant, even though they have descended from resistant third generation parents.

The hybrids of Monarch and Hull-less give a reaction to the loose smut similar to that of the hybrids of Early Gothland and Hull-less to the covered smut. In these hybrids also there is a rather high percentage of infection (31.3 percent) in the second generation, 415 F_2 plants being inoculated, of which 130 were infected. These results may be interpreted as fitting fairly well the three to one ratio. In the third generation, however, we find too few pure resistant families and too many segregating and susceptible combined. The F_3 families have descended from both inoculated and uninoculated F_2 plants. In the former group we would not expect any susceptible families unless the parental F_2 plant was a chance escape. On the other hand, if the F_3 progenies descended from uninoculated F_2 plants, we would expect the three types of families, resistant, segregating, and susceptible, in the proportion of 1 : 2 : 1. These results are far from being realized. It may be noted, however, that a large proportion of the so-called susceptible F_3 progenies have given a percentage of infection only slightly above 50 percent.

In the fourth generation we get still further interesting data. We find a number of susceptible individual plants in a great many fourth generation progenies, although they have descended from resistant F_3 progenies and, in many cases, also from resistant F_2 parents. These results are quite in contrast to those obtained with such hybrids as Early Gothland × Victor and Hull-less × Black Mesdag, in which resistant F_2 and F_3 generations have given pure resistant F_4 and later generations.

The hybrids Early Gothland and Hull-less, to loose smut, and Monarch and Hull-less, to covered smut, have shown a behavior entirely similar to the parental varieties. In both hybrids high susceptibility has been shown in both the F_2 and the F_3 generations.

SUMMARY

This paper presents the results with two different sets of hybrids—Early Gothland with Hull-less, and Monarch with Hull-less. Early Gothland is susceptible to the loose smut and resistant to the covered, Monarch is exactly the reverse in its behavior, and Hull-less is susceptible to both smuts. Data have been obtained on the second, third, and fourth generations of the hybrids.

I. Hybrids 19 and 20, Early Gothland × Hull-less.
  1. Results with the F_2 generation.
    A. There were 90 F_2 plants inoculated with *Ustilago Avenae* and 85 (94.4 percent) were infected.
    B. There were 191 F_2 plants inoculated with *Ustilago levis* and 88 (46 percent) were infected.
  2. Results with the F_3 generation.
    A. F_3 progenies inoculated with *Ustilago Avenae*.
      a. F_2 plant uninoculated. There were 24 progenies containing 455 plants, all of which were infected.
      b. F_2 plant inoculated with covered smut. There were 24 progenies of this group containing 460 plants, of which 457 (99.3 percent) were infected.
      c. F_2 plant inoculated with the loose smut. There were 4 progenies of this group, containing 53 plants, of which 50 (94.3 percent) were infected.
    B. F_3 progenies inoculated with *Ustilago levis*.
      a. F_2 plant uninoculated. There were 46 progenies of this group, 4 of which were resistant, 17 gave percentages of infection below 50 percent, and 25 percentages between 50 and 100 percent.
      b. F_2 plant inoculated with covered smut. There were 92 progenies, 18 of which were resistant, 56 gave percentages of infection below 50 percent, and 18 gave percentages above 50 percent.
  3. Results with the F_4 generation.
    There were 88 F_4 progenies descended from resistant F_3 progenies inoculated with the covered smut. In 26 of these progenies one, occasionally two, plants were infected. There were 18 F_4 progenies descended from 3 different F_2 plants which showed complete resistance in the second, third, and fourth generations.

II. Hybrid 24, Monarch × Hull-less, and Hybrids 25–27, Hull-less × Monarch.
  1. Results with the F_2 generation.
    A. There were 148 F_2 plants inoculated with *Ustilago levis* and 143 (96.6 percent) were infected.

B. There were 415 F_2 plants inoculated with *Ustilago Avenae* and 130 (31.3 percent) were infected.

2. Results with the F_3 generation.

A. F_3 progenies inoculated with *Ustilago levis.*

a. F_2 plant uninoculated. There were 48 progenies containing 854 plants, of which 832 (97.4 percent) were infected.

b. F_2 plant inoculated with loose smut. There were 48 progenies containing 844 plants, of which 826 (97.8 percent) were infected.

c. F_2 plant inoculated with covered smut. There were 4 progenies containing 51 plants, of which 47 (92.1 percent) were infected.

B. F_3 progenies inoculated with *Ustilago Avenae.*

a. F_2 plant uninoculated. There were 90 progenies, 9 of which were pure resistant, 51 gave percentages of infection less than 50 percent, and 30 percentages of infection greater than 50 percent.

b. F_2 plant inoculated with loose smut. There were 261 progenies, of which 54 were resistant, 181 gave less than 50 percent infection, and 26 more than 50 percent infection.

3. Results with the F_4 generation.

There were 184 F_4 progenies descended from resistant F_3 progenies inoculated with the loose smut.

A. Of these F_4 progenies, 48 descended from 8 uninoculated F_2 plants, and 24 showed one or more smutted plants. A total of 1063 plants were grown and 52 were infected. The highest percentage of infection in any progeny was 20.8 percent.

B. The remaining 136 progenies descended from 23 F_2 plants which had been inoculated with loose smut, and 62 of these contained one or more infected plants. The total number of plants was 3069, and the total number infected was 150. The highest percentage of infection in any F_4 progeny was 39.1 percent.

III. The results with the hybrids of Early Gothland × Hull-less with the covered smut contrast sharply with those obtained with the hybrid Early Gothland × Victor, although both hybrids furnish a similar combination of resistance of the parents to both smuts. In the hybrids of Early Gothland × Hull-less there is a great preponderance of susceptibility in the F_2, F_3, and F_4 generations, while in the hybrid Early Gothland × Victor there is a preponderance of resistant F_2 plants and F_3 progenies.

IV. The Hybrids of Monarch and Hull-less have behaved towards the loose smut in a manner corresponding to the behavior of Early Gothland and Hull-less to the covered smut. There is a small excess of susceptible F_2 plants, and the preponderance of susceptible and segregating progenies in the F_3 and F_4 generations is conspicuous.

V. The hybrids of Early Gothland × Hull-less in the F_2, F_3 and F_4 generations were as susceptible to the loose smut as were their parents, Early Gothland and Hull-less.

VI. The hybrids between Hull-less and Monarch in the F_2, F_3 and F_4 generations were as susceptible to the covered smut as were their parents, Monarch and Hull-less.

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REGENERATION AND INDUCED POLYPLOIDY IN FERNS[1]

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INTRODUCTION

Induced apospory, or the regeneration of prothalli from the young leaves of ferns, has been known since 1908, when Goebel (8) described the appearance of prothallial tissue on the detached primary leaves of members of the Polypodiaceae and the Cyatheaceae. *Pteris longifolia* regenerated prothalli from the surface of the leaf and from the petiole. Rhizoids and sex organs were present on the prothalli, but sporophytes never appeared. *Ceratopteris thalictroides* produced prothallus-like structures which bore rhizoids and antheridia, and also stomata near the antheridia. Some leaves of this same fern developed buds on the petioles from which there arose structures intermediate between leaves and prothalli. *Gymnogramme chrysophylla* also produced prothallus-like outgrowths which contained stomata. When the primary leaves of *Polypodium aureum* were cut off, sporophytes grew from the buds produced on the epidermal cells. *Alsophila van Gertii* produced adventitious buds on the leaves, typical prothalli, and intermediate structures such as prothalli containing stomata. Later Goebel (9) described regeneration from a third family. Primary leaves of *Osmunda regalis* developed typical prothalli bearing rhizoids.

Woronin (25) made similar experiments with the leaves of some apogamous ferns as well as with some which reproduced sexually. *Trichomanes Kraussii* of the Hymenophyllaceae was apogamous and its leaves developed prothalli by apospory, especially if they were cut off and kept growing on a suitable substratum. The apospory could go still further in that the border cells of the leaves produced antheridia. This condition was termed "apoprothallia." Two species of *Pellaea*, both of which were apogamous, were capable of regenerating. *Pellaea nivea* produced filamentous prothallus-like outgrowths from the surface cells of detached leaves. Only the primary leaves of *Pellaea flavens* regenerated; but sometimes the secondary leaves

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which were cut off continued to grow until they were much larger, and the blade was very irregular in outline. The primary leaves often grew in the same manner and they produced other structures as well. Numerous rhizoids developed on the petioles of some of the leaves, while others produced adventitious buds. Two non-apogamous ferns reacted in much the same manner. Buds which gave rise to leaves and roots developed both on the blade and on the petiole of *Notochlaena Marantae*. Small prothalli bearing rhizoids and antheridia developed from the petiole of a detached leaf of *Gymnogramme farinifera*.

Brown (3) obtained regeneration from the petiole of a leaf of *Phegopteris polypodioides*. A cellular mass resembling a prothallus was formed and from it rhizoids developed, as well as true leaves and structures intermediate between prothalli and true leaves.

Heilbronn (10) described the formation of rhizoids and sporophytic buds on detached leaves of *Cystopteris fragilis*.

Many of the regenerating leaves described by Goebel and Woronin developed structures composed of both prothallus-like cells and cells which were typically sporophytic, such as tracheids and stomata. Lang (12) has described some extreme instances of the production of sporophytic and gametophytic structures without definite sequence. The prothalli of Lang's cultures were grown from spores. *Scolopendrium vulgare* var. *ramulosissimum* and *Nephrodium dilatatum* var. *cristatum gracile* produced sporangia on the prothalli, sometimes apparently from transformed archegonia Archegonia were present on the same prothalli with sporangia; and reproduction was both apogamous and sexual. Lang (14) reported a similar situation in *Scolopendrium vulgare* var. *ramo-digitatum*. Some of the sporangia produced spores which seemed to be mature, but they did not grow.

Pace (19) reported the presence of sporangia on prothalli of a fern which was probably one of the Osmundaceae.

Since the literature relating to apospory and apogamy in general has been reviewed by a number of other writers it is not discussed here (Allen, 1; Ernst, 5; Farmer and Digby, 7; Steil, 23).

In all the earlier works on induced apospory in ferns the regenerated prothalli did not develop beyond the production of sex organs. In 1925 Heilbronn (11) reported a study of *Polypodium aureum*, the primary and secondary leaves of which regenerated to form adventitious buds, filamentous prothalli, and typical heart-shaped prothalli. Tetraploid sporophytes developed from the regenerated prothalli which differed from the diploid plants not only in chromosome number but also in cell size.

In addition to the studies on regeneration in the ferns, the Bryophytes have been the subject of many similar experiments. Gametophytes were produced from sterile cells of capsules of *Anthoceros* by Lang (13) and by Schwarzenbach (21), but sporophytes were not obtained from these re-

generated structures. Éliè and Émile Marchal (16–18), Wettstein (24), and LaRue (15) have induced apospory in various species of mosses. The Marchals produced tetraploid sporophytes bearing diploid spores. In some species tetraploid gametophytes were obtained by regeneration from tetraploid capsules. By regeneration Wettstein obtained bivalent, trivalent, and quadrivalent races from capsules having the $2x$, $3x$, and $4x$ chromosome numbers. Octoploid gametophytes were produced from capsules of intergeneric hybrids.

Polyploidy in ferns has not been as extensively investigated as has polyploidy in the mosses. With the exception of the tetraploids developed by Heilbronn (11) in a single species, no fern sporophytes with more than two complete sets of chromosomes have been produced experimentally. The apparent neglect of studies on the ferns has probably been due rather to the difficulty of the material than to any lack of interest.

It seemed desirable to extend the work along different lines and this paper deals with attempts to solve a number of problems, some of which have been suggested by similar investigations in the mosses. Attempts were made to induce apospory and the production of tetraploid sporophytes in as many species as possible, and to investigate the conditions under which regeneration occurred. Since the tetraploid capsules of mosses regenerated gametophytes, it was believed that prothalli with the $4x$ chromosome number could be produced from leaves of tetraploid sporophytes of ferns. The present work deals, also, with attempts to produce triploid sporophytes by crossing $2x$ gametes and x gametes. It was desirable to show that fertilization was necessary for the production of sporophytes on regenerated gametophytes and to demonstrate fertilization in the $2x$ prothalli. Apospory and apogamy as they occur in nature are closely related to events resulting from the cultivation of detached leaves of young sporophyte plants; and the further study of induced apospory may throw some light on abnormalities in life cycles. Further evidence that apogamy does not necessarily follow induced apospory was needed.

Methods

Cultural Procedure

Since all prothalli and young sporophytes were obtained by growing them under controlled conditions, the success of the entire investigation depended on the cultural methods and the technique of handling the material. Therefore the development of the method will be described in greater detail than would otherwise be necessary.

Leaves or parts of leaves with ripe sporangia were thoroughly washed under running water in order to remove foreign fern spores and mold spores; then washed several times in sterile distilled water and put in sterile Petri dishes to discharge their spores. When they were planted they were

transferred with sterile instruments. The medium which was found to be most satisfactory for nearly all of the ferns grown was .12 percent Shive's nutrient solution. All solutions were sterilized in the autoclave.

The spores were planted in small glass capsules on the solution and the cultures were kept in a greenhouse where they never received direct sunlight. The spores germinated to form prothalli floating on the surface; and the young prothalli were transferred to fresh solution every three or four weeks and thinned out in order to give them plenty of room to develop. In this way they grew to maturity and formed sex organs and young sporophytes. Usually some of the sex organs were immersed in the solution so that fertilization took place; but if the sporophytes were slow to form, especially in ferns where the antheridia are not produced in such large numbers, shaking the dish brought about the opening of the sex organs and fertilization.

The time required for germination varied from two days to four weeks according to the species and the age of the spores. Young sporophytes with one or two leaves ready for regeneration experiments were formed from two to five months after the spores were planted, the time varying with different ferns.

If the prothalli showed a tendency to sink, especially after they had been disturbed in order to bring about fertilization, they grew better if transferred to capsules containing three quarters of an inch of sterilized white sand soaked with the nutrient solution.

The common contaminations were algae and fungi, although washing the spore-bearing leaves usually removed most of the mold spores. Numerous collections were made at different times and from different parts of the plant; and each collection was preserved in separate, sterile Petri dishes. The collections which were found to be contaminated could be discarded after the first trial plantings. In the early stages of the work methods of control were tried which would rid the cultures of contaminations. A very weak solution of copper sulfate would sometimes kill algae without injuring the prothalli; and a weak solution of potassium permanganate would often free the cultures from fungi. However, a better method was to grow large numbers of capsules of the prothalli in order that any which became contaminated could be discarded. All cultures were kept in a clean place in the greenhouse. When a dish was to be examined, the outside was wiped off with alcohol before it was opened.

The above culture method was used for all the ferns except *Scolopendrium* and *Pteris*. Knopf's nutrient solution was found to be better for *Scolopendrium*. *Pteris* was grown on sterilized sphagnum soaked with Shive's nutrient solution. Agar made with a .24 percent Shive's solution was used in experiments to prevent fertilization. Prothalli with immature archegonia were transferred from solution to Petri dishes containing agar. The prothalli grew to be very large and produced numerous archegonia if they were transferred to fresh agar every two or three weeks.

Contaminations were more common on agar than on solution, and they often appeared soon after the prothalli were transferred to fresh agar. Frequent sterilization of the needles in a flame during the process of removing the prothalli to a dish of fresh agar was necessary. When a dish containing prothalli to be transferred was brought from the greenhouse, the surface was washed with alcohol to remove sources of contamination.

In all the species regeneration was brought about by cutting the young leaves off with sterile needles, and transferring them to fresh .12 percent Shive's solution. The leaves usually floated on the surface; but if there was a tendency to sink, they were supported by sand. From ten to twenty-five leaves were placed in each capsule containing approximately 30 cc. of the sterile solution. The first, second, and third leaves were cut off as they were formed on the embryo; but regeneration was not very common in the third leaves. Wounding seemed, in some cases, to induce regeneration since the prothalli often developed where the leaf had been injured in cutting. Regeneration usually took place within two to four weeks after the leaves were cut off, but in a few species the leaves remained green for three months before regeneration started.

It was necessary to exercise great care in the preparation of leaves for regeneration in order to prevent contaminations. All apparatus and solutions were sterilized, and the leaves were cut and transferred as quickly as possible. Good results were never obtained in any culture contaminated with green algae or blue-green algae. The nutrient solution was a very favorable place for algae, which grew much faster than the tissue regenerated from the leaves. Since the regenerated prothalli formed in contaminated cultures were always crowded out by the algae and died in an early stage, it was important to prevent contaminations and to remove all contaminated capsules from the greenhouse immediately. Mold contaminations were not so harmful since conditions were not favorable for the growth of fungi, and they usually died in a few weeks. All the leaves which died without regenerating were removed because fungi could grow on them. It was often necessary to transfer all the green leaves to clean, sterile dishes of solution when some of the leaves died and became moldy.

In a few cultures where algae appeared after the regenerated prothalli were well established, the *2x* prothalli were saved by transferring them to soil. The soil was not sterilized, since the prothalli grew better on unsterilized soil. Contaminations by fern spores were controlled by keeping the pots of soil moist and under glass covers for several weeks. Under such conditions, any foreign fern spores would germinate and could be removed before the *2x* prothalli were put on the soil. All prothalli grown on soil were kept under glass covers, and were either watered from below or with sterile water.

Technique

The cell measurements were made with an ocular micrometer on material mounted in lactic acid according to the method described by Simpson (29). The leaves or prothalli were kept in lactic acid in a warm place until they became clear and then mounted. The areas of the epidermal cells were obtained by measuring camera lucida drawings of the cells with a planimeter. The actual area of the cell was determined by dividing the area derived from the planimeter measurement by the magnification of the drawing. The areas of the prothallial cells were obtained by multiplying the lengths by the widths since the cells were approximately rectangular.

The sporogenous tissue used for chromosome counts in *Aspidium marginale* was fixed in Flemming's medium solution. All other material for chromosome counts was fixed in formol-acetic-alcohol with the following formula: 50 percent alcohol, 100 cc.; formalin (neutralized by magnesium carbonate), 7 cc.; glacial acetic acid, 7 cc.

The sections were cut six microns in thickness and stained with Flemming's triple stain or with gentian violet alone according to Newton's method.

Experiments

Regeneration was tried in sixteen different species of ferns from the Polypodiaceae and the Osmundaceae, but regenerated structures were never obtained from five of this number, as follows; *Osmunda Claytoniana* L., *Onoclea sensibilis* L., *Camptosorus rhizophyllus* (L.) Link., *Aspidium spinulosum* var. *intermedium* (Muhl.) D.C. Eaton, and *Pteris aquilina* L. Since the numbers of leaves of the five ferns used in these cultures were small, it is impossible to draw any conclusions as to their ability to produce prothalli by induced apospory.

Several other ferns which did not prove suitable for an extensive study formed prothalli by induced apospory. *Scolopendrium vulgare* Sm. and *Dicksonia punctilobula* (Michx.) Gray formed outgrowths from the tips of the lobes of the primary leaves. It was difficult to determine where the sporophytic tissue of the leaf ended and the gametophytic tissue of the prothallus began in some of these structures. Sometimes filamentous prothalli similar to those described for *Woodsia obtusa* were present. No sex organs were formed and none of the regenerated tissue lived longer than a few weeks.

Polystichum acrostichoides (Michx.) Schott regenerated heart-shaped prothalli which bore antheridia. These prothalli grew for more than a year and became very large but had never developed archegonia when they became contaminated with green algae and died. The prothalli grown from spores produced few archegonia in these cultures, even when they had grown for a long time and were very large.

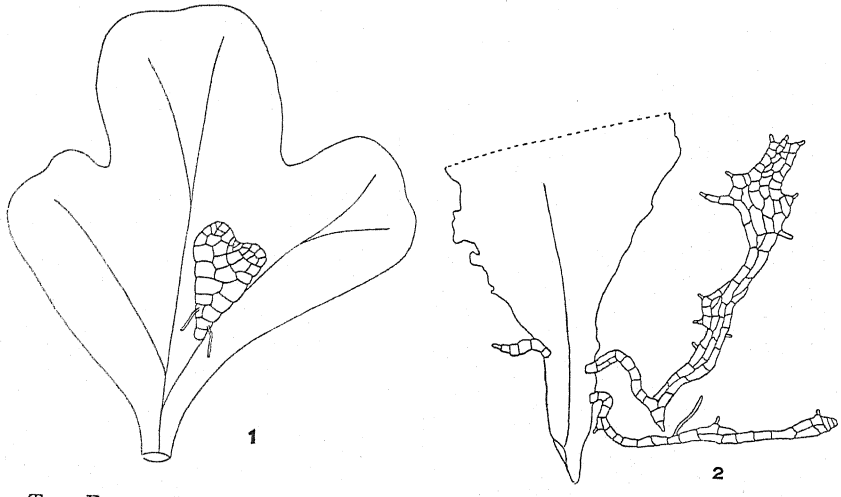
Asplenium platyneuron (L.) Oakes regenerated from primary leaves, secondary leaves, and roots. Both the leaves and the roots developed

filamentous prothalli, heart-shaped prothalli, and sporophytic buds. Antheridia were borne on the heart-shaped prothalli but no archegonia were present when this culture became contaminated with green algae and died.

Cystopteris fragilis (L.) Bernh., *Osmunda regalis* L., and *Athyrium filix-femina* var. have regenerated heart-shaped prothalli with archegonia and antheridia from which sporophytes developed. A report of the studies on these three ferns will be published later.

Pteris cretica var. albo-lineata Hook.

The spores of this fern were obtained from the commercial florist J. F. Anderson, Short Hills, New Jersey. They were planted in glass capsules on a medium of sterilized sphagnum soaked with Shive's nutrient solution. The gametophytes developed very quickly, forming heart-shaped prothalli, but never bearing archegonia. Six weeks after the spores were planted, nearly all the prothalli formed apogamous sporophytes in the manner described by Farlow (6) and deBary (2) for *Pteris cretica*. While the sporophytes were quite young (having only two or three leaves) and while they were still growing on the sphagnum, the primary leaves of about thirty were cut off and put on Shive's solution. Five weeks after the



TEXT FIG. 1. *Pteris cretica* var. *albo-lineata*. Primary leaf regenerating a heart shaped prothallus.

TEXT FIG. 2. *Woodsia obtusa*. Leaf with three regenerated filamentous prothalli.

leaves were cut off, regeneration had begun on the dorsal surface of one leaf near a vein. Very soon the regenerated tissue formed rhizoids and broadened out to form a heart-shaped prothallus (text fig. 1). The development of this prothallus resembled the development of the prothalli of *Pteris cretica* var. *albo-lineata* grown from spores. No archegonia were formed on the regenerated prothallus, and no antheridia were observed.

About eight weeks after this prothallus was regenerated, it produced an apogamous sporophyte in the same manner that the original apogamous sporophytes in this species were developed. This apogamous sporophyte grew for several months and produced a number of leaves and roots. The regeneration described above was the only case in *Pteris cretica* var. *albo-lineata*.

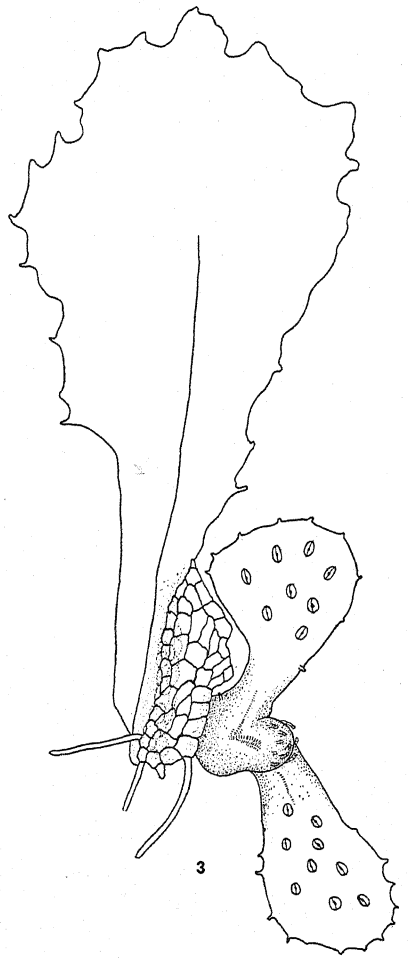
Woodsia obtusa (Spreng.) Torr.

Spores of *Woodsia obtusa* were collected from a plant growing in cultivation near Cold Spring Harbor, Long Island. They were planted on Shive's solution, and the young sporophytes developed on the gametophytes floating on the solution. Approximately 1500 leaves, 75 percent of which were primary leaves, were cut off to induce regeneration; 6.25 percent of the leaves regenerated but no normal heart-shaped prothalli were ever formed. No archegonia developed on any of the regenerated tissue, and probably no antheridia, since none were ever found although a large number of the prothalli were examined carefully. There were two different types of regeneration: filamentous prothallus-like structures, and sporophytes with true leaves and roots. The two kinds of structures were often found on the same leaf and they developed either from the petiole or from the blade.

The filamentous structures consisted of undifferentiated cells similar to normal prothallial cells and bore rhizoids (text fig. 2). They never became very large and usually lived only a few weeks. Text figure 3 shows a young sporophyte developing at the base of the petiole of a leaf which had been cut off and cultivated on solution for more than two months. The shape of the leaf was modified by growth in the marginal region, giving it a very irregular outline. Growth of this type occurred more often in leaves which were cut off before they had reached full size. On the leaf shown in text figure 3, prothallial tissue was formed before the regenerated sporophyte developed. Two leaves and a growing point grew from the mass of cells where the prothallus was attached to the petiole, the growing point later giving rise to leaves and roots. This illustration shows the development of a prothallus followed by the development of true leaves. Other regenerating leaves developed prothallial tissue first, then a series of structures intermediate between prothalli and leaves, and finally true leaves.

Regeneration in *Woodsia* took place in the roots also. Plants grown in glass capsules on a clear solution develop chlorophyll in the roots, especially in the meristematic tissue at the tip. When the green roots were cut off and kept in nutrient solution for two or three months, they began to regenerate. The tissue of the root tip within the root cap became greener and grew, forming a spherical mass which broke through the root cap. This mass of cells developed further in much the same manner as the stem initial in a normal embryo, and formed leaves and roots. The young sporophyte remained attached to the root which regenerated it. It resembled a sporophyte developed from a fertilized egg; and when it was

transferred to soil, it grew to maturity. Nearly all the regenerating roots formed sporophytes in the manner described; but a few formed filamentous prothalli which were similar to those formed on the regenerating leaves. They developed from the root tip or from the cells a short distance from the tip. The writer is not aware that regeneration of prothalli from roots has been described previously, although the formation of sporophytes on uninjured roots or on detached roots of *Ophioglossum* has been described



TEXT FIG. 3. *Woodsia obtusa*. Sporophytic bud formed on prothallial tissue regenerated from the petiole of a leaf. Leaf showed growth in the marginal region.

by Campbell (4) and by Poirault (20). The roots of *Woodsia* regenerated the same types of structures as the leaves of that species.

Shive's and Knopf's solutions were both used in the regeneration of *Woodsia*. Two series of solutions of different concentrations varying from

.3 grams per liter to 5 grams per liter were tried in order to test the effect of the medium. About 200 detached leaves were cultivated on the different concentrations of each solution. The two solutions gave approximately the same results in regard to the number of leaves which regenerated and the structures produced; but contaminations by algae were more common in Knopf's solution. Both filamentous prothalli and sporophytic buds were formed in all the concentrations of both solutions. The number of sporophytes was slightly larger in the higher concentrations of both solutions; and in general, more leaves regenerated in the solutions of medium concentrations. Heart-shaped prothalli were never formed and all the regenerated tissue either died or formed sporophytes capable of developing to maturity.

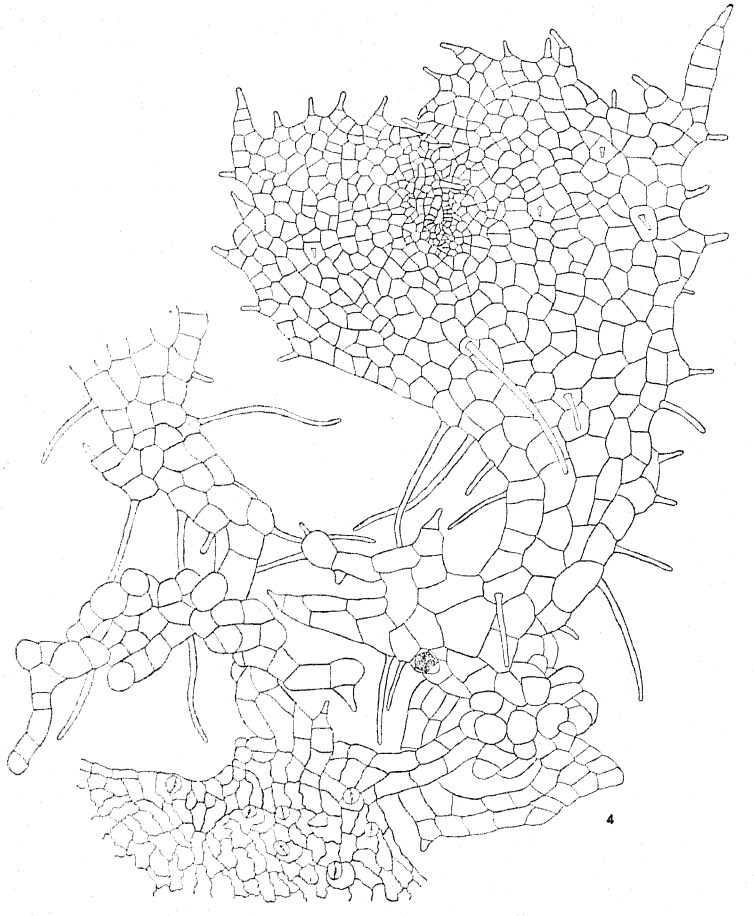
Aspidium marginale (L.) Sw. (Long Island)

The spores of *Aspidium marginale* were collected at Cold Spring Harbor, Long Island, New York. Regeneration in this species took place readily, usually about four weeks after the leaves were cut off, and 33 percent of the 135 leaves which were cut regenerated. Growth began in the superficial cells of the blade either on the surface or on the margin as shown in text figure 4. In *Aspidium* it was noticed that regeneration often took place near an injured spot on the leaf. Only one case of the formation of a sporophytic bud as described for *Woodsia* was found and in this instance the regeneration was from the petiole.

The Regenerated Gametophytes

The first structures formed were filamentous, usually only one or two cells wide, and they bore rhizoids and a few antheridia. A growing point was very soon established in each and a true heart-shaped prothallus developed which grew to sexual maturity very quickly. The development after the short filamentous stage was not observed to differ from the normal development of a prothallus from a spore. The regenerated prothalli resembled the normal in that they were typically heart-shaped with the growing point in the notch and in that they produced archegonia on the thickened cushion and antheridia and rhizoids on the posterior part; but when cell measurements were taken it was found that the cells were larger. With the exception of the $4x$ prothallus, which is discussed below, the measurements were made on cells in the wings of sexually mature prothalli all of which had been grown under like environmental conditions. Gametophytes vary under different external conditions, such as differences in substratum and in the amount of light. Also under crowded conditions normal development is prevented; the prothalli are smaller and the cells themselves are smaller. Therefore the x and the $2x$ prothalli were grown on the same solution and under the same conditions as to light and temperature; and those on which the cell measurements were taken were chosen from cultures having approximately the same number of plants to a given

amount of nutrient solution. Normal and regenerated prothalli were selected which were nearly the same size, and which bore sporophytes at the same stage of development. The length and the width of each of four hundred cells was measured from both the normal and the regenerated prothalli and the variates were taken from a number of different plants. The results of the measurements of the prothallial cells are shown in table 1.



TEXT FIG. 4. *Aspidium marginale.* Heart shaped prothallus regenerated from the margin of a leaf. Filamentous prothalli were formed first.

The length of the *2x* cells did not show much increase but it was significant since the difference in lengths was six times the probable error. The width showed a much greater increase and the difference in widths was twenty-seven times the probable error.

Chromosome counts were made from cells of the developing antheridia

TABLE I. *Cells of Prothalli of Aspidium marginale (Long Island)*

Prothallus	No. Cells Measured	Lengths in μ	Difference from (*a*)	% Increase over (*a*)
(*a*) *x*	400	117.19 ± .87		
(*b*) 2*x*	400	125.47 ± 1.06	8.28 ± 1.37	7
(*c*) 4*x*	100	112.53 ± 1.61	4.66 ± 1.83	
		Widths in μ		
(*a*) *x*	400	49.97 ± .24		
(*b*) 2*x*	400	62.67 ± .39	12.70 ± .46	25.4
(*c*) 4*x*	100	90.32 ± 1.16	40.35 ± 1.18	80.0
		Areas in sq. μ		
(*a*) *x*		5900		
(*b*) 2*x*		7900		34
(*c*) 4*x*		10200		73

in both the normal and regenerated prothalli. The number in the prothalli grown from spores was 36 ±, while 65 ± chromosomes were found in the regenerated prothalli. The dividing cells were rare, and it was necessary to section many prothalli bearing young antheridia in order to find a sufficient number of cells in the proper stages to make chromosome counts. A number of counts were made; but, due to the large number of chromosomes and the fact that they are long and slender, it is almost impossible to be sure of the exact number from counts made in somatic tissue. It seems certain from the above numbers that the diploid number is carried over into the regenerated prothalli.

Observations were made on mature sex organs and by means of paraffin sections on various stages in their development. Some antheridia on the regenerated gametophytes produced 32 sperms while others bore 64; the same numbers were observed in various antheridia on the normal prothalli. The antheridia of the 2*x* prothalli were seen to open; the sperms were motile and were attracted to the archegonia; sperms were found in the necks of the archegonia sectioned in paraffin. The archegonia did not differ from the normal ones except perhaps by a slight increase in size. Unopened archegonia were found showing the egg, the ventral canal cell, and the neck canal cell with its two nuclei.

Although fertilization has not been found in this species, there is reason to believe that it took place in a normal fashion. Fertilization was found in the 2*x* prothalli of another fern which is described below; and it is to be expected that it will be demonstrated in this species also.

The Sporophytes Produced by the Diploid Gametophyles

Sporophytes were formed about four months after the regenerated prothalli developed; and from the first it seemed probable that they were the result of fertilization, because they developed a few weeks after the prothalli had been shaken up in the solution on which they were growing. Since the 2*x* prothalli were grown on sterile media, and in dishes with lids,

there was no possibility of contamination by other ferns. The sporophytes developed from archegonia because the young embryos were observed in the enlarged venters with the brown necks of the archegonia still attached; and the sections showed a well developed foot. No record has been found in the literature that apogamy or parthenogenesis occurs in the normal *Aspidium marginale*. Paraffin sections of *2x* prothalli bearing archegonia and young embryos showed that the course of development was normal. Two celled embryos showed that the first cell wall was parallel to the axis of the neck of the archegonium as in the normal embryos. Four, eight, and sixteen celled embryos were found as well as later stages.

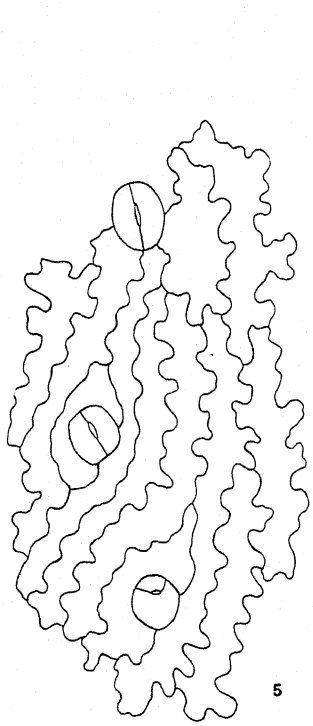
Due to the rapid growth of prothalli and their tendency to regenerate smaller prothalli, a large number of *2x* gametophytes was available for an experiment on fertilization. The prothalli grown on sand or on solution never became very large without producing embryos, there being opportunities for fertilization to take place every two or three weeks when they were transferred to fresh solution, and the sex organs in contact with the solution opened. Therefore a number of small prothalli either with no archegonia or with very small ones were removed to agar. In a few weeks numerous mature archegonia were present on all the prothalli on agar; and they continued to form in large numbers, as the prothalli grew to be several times as large as those growing on sand or on solution. About 150 prothalli were kept on agar for periods varying from one to four months. Only ten sporophytes appeared on these gametophytes and they probably developed because a few drops of water condensed on the lids of the Petri dishes and dropped on the prothalli. The small number of sporophytes formed on agar, contrasted with the fact that all prothalli on sand or solution produced embryos, made it seem highly probable that all the sporophytes were the result of fertilization.

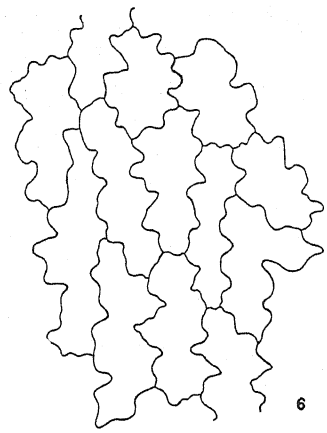
In order to obtain more direct evidence that fertilization was necessary for the formation of sporophytes, a dish containing about fifty prothalli with mature sex organs was filled with nutrient solution for twenty-four hours. Other dishes of similar prothalli with mature sex organs were not filled with solution. After five weeks forty-three young sporophytes were counted in the dish which had been filled with solution. All the sporophytes were approximately of the same size, showing that the archegonia had all been fertilized at the same time. The other dishes with prothalli having mature sex organs which had not been in solution did not develop sporophytes.

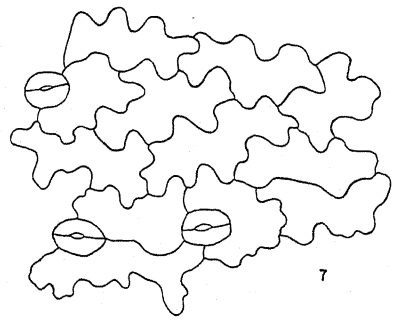
The evidence from the chromosome numbers strengthens the theory that fertilization took place. Chromosome counts were made in the cells of the first leaf of the embryo where mitotic figures were abundant. The very large number of chromosomes made it impossible to be sure of the exact number, although numerous careful counts were made on a large number of cells. It seemed certain that at least 120 chromosomes were

present, since the counts varied from 120 to 130 in different cells. When this number was compared with the numbers found in the gametophytes there was every reason to believe that the sporophytes formed on the regenerated prothalli were tetraploids.

A comparison of the young diploid and tetraploid sporophytes showed some differences. The leaves of the tetraploid were more irregular in outline, in some cases showing peculiar thickenings and foldings on the







TEXT FIG. 5. *Aspidium marginale.* Lower epidermal cells of tetraploid sporophyte.
TEXT FIG. 6. *Aspidium marginale.* Upper epidermal cells of tetraploid sporophyte.
TEXT FIG. 7. *Aspidium marginale.* Lower epidermal cells of diploid sporophyte.

surface. A microscopic study of the fully developed primary leaves of diploid and tetraploid plants at the same stage of development showed variations in size of stomata, and variations both in the size and shape of the epidermal cells (text figs. 5, 6, 7, and 8). Table 2 shows the numbers of measurements made and the sizes of the cells, in microns, of the diploid

and tetraploid plants. A number of plants were used for all the measurements of the different kinds of cells.

TABLE 2. *Cells of Leaves of Aspidium marginale (Long Island)*

Sporophyte	No. Cells Measured	Stomata Lengths in μ	Difference	% Increase
$2x$	300	42.77 ± .19		
$4x$	300	54.11 ± .25	11.34 ± .32	26
		Upper Epidermis Lengths in μ		
$2x$	200	101.59 ± 1.07		
$4x$	200	129.14 ± 1.26	27.55 ± 1.65	27
		Lower Epidermis Lengths in μ		
$2x$	350	119.45 ± 1.11		
$4x$	350	233.22 ± 2.18	113.77 ± 2.44	95
		Lower Epidermis Widths in μ		
$2x$	300	60.61 ± .49		
$4x$	300	63.56 ± .61	2.95 ± .78	4.8
		Areas in sq. μ Lower Epidermis		
$2x$		3952		
$4x$		6916		75

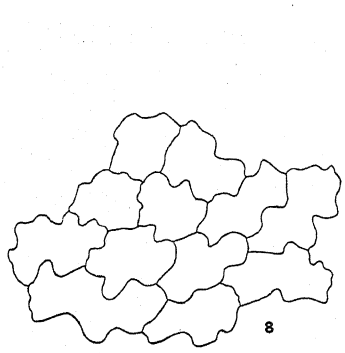
The lengths of two hundred lower epidermal cells of full grown leaves of a mature plant were measured. The mean was 119.62 ± .92 microns. When this was compared with the length of the corresponding cells of normal primary leaves it was found that the difference was less than the probable error. Therefore it is to be expected that the cells in the mature tetraploid sporophytes will be approximately of the same size as the cells of the primary leaves of tetraploid sporophytes.

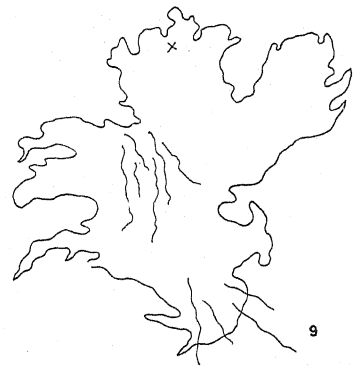
The difference in widths of the lower epidermal cells of the $4x$ and $2x$ plants is only 3.8 times its probable error. In all other measurements the differences are so great that there can be no question of their significance. By means of a planimeter the areas of twelve cells of the lower epidermis of the diploid plant, and the areas of the same number of cells of the tetraploid plant, were measured from camera lucida drawings. Cells were selected whose measurements corresponded on the average to the dimensions given in table 2. From the figures thus obtained the areas of single cells in square microns were computed.

In his description of the tetraploid plants of *Polypodium aureum*, Heilbronn (11) states that the areas of normal and tetraploid epidermal cells are to each other as 1 : 2.2. Therefore the epidermal cells of tetraploid plants of *Aspidium marginale* do not show as great an increase in size as the epidermal cells of *Polypodium aureum*.

Regeneration from Tetraploid Sporophytes

Regeneration was also obtained from leaves of the $4x$ sporophytes formed on the regenerated prothalli described above, giving prothalli with the $4x$ chromosome number. The $4x$ regeneration resembled the early stages of the $2x$, the gametophytes being filamentous and without a growing point, and sometimes developing fifty or sixty cells in a single row. One culture of $4x$ tissue obtained from a single leaf produced twelve or more prothalli at the end of nine months. Very few of these prothalli were truly





TEXT FIG. 8. *Aspidium marginale.* Upper epidermal cells of diploid sporophyte.
TEXT FIG. 9. *Aspidium marginale.* Prothallus regenerated from leaf of tetraploid sporophyte.

heart shaped although sex organs of both kinds were produced regardless of the shape of the prothallus. Structures similar to that shown in text figure 9 were common, having many segments which often ended in filaments of a single row of cells. The region marked "x" consisted of cells which had not reached full size and the notches in this lobe resembled growing points. A number of archegonia developed on a broad, irregularly ribbon-shaped prothallus which had developed a thickened region similar to a cushion along one edge, while the other side consisted of a single layer of cells like the wings of a normal prothallus.

One hundred measurements of the length and width of cells of the prothallus shown in figure 9 were made. The mean length was 112.53 $\pm$ 1.61 microns and the width was 90.32 $\pm$ 1.16. Since the measurements were taken from a single prothallus, due to lack of material, and since that prothallus had not developed sex organs and was probably not at exactly the same stage of development as the other prothalli whose measurements are given in table 1, a comparison of the results obtained in the two instances may be misleading. However, it seems to be evident that the width of the $4x$ prothallial cells is greater than that of the $2x$ cells. A similar difference was observed in the comparison of the $2x$ prothallial cells with the ones having the x chromosome number.

The sex organs on the $4x$ gametophytes seemed to be normal but a more careful examination must be made when material is available. Although the same $4x$ tissue has been kept in culture for nearly a year, very few prothalli have developed because growth is very slow. It is hoped that prolonged culture will show that $4x$ prothalli can produce sporophytes with the $8x$ chromosome number.

Aspidium marginale (L.) Sw. (Pennsylvania)

Spores of *Aspidium marginale* were also collected from a plant which had been brought from Penowa, Pennsylvania, and was growing in the Botanical Gardens at the University of Michigan. They were grown in order to repeat the experiments made with the same species collected on Long Island.

Chromosome counts made at sporogenesis showed that the haploid number was either 38 or 39. The similarity in the results of the experiments on the cultures from the two ferns suggests that they may be similar in constitution and that the chromosome numbers in the plant from Long Island may be

$$x = 38, \qquad 2x = 76, \qquad 4x = 152,$$

instead of

$$x = 36 \pm, \qquad 2x = 65 \pm, \qquad 4x = 120\text{–}130,$$

as obtained from the counts made in the vegetative divisions of the Long Island plant.

Approximately 800 leaves were detached from the young embryos of *Aspidium marginale* (Pennsylvania) and placed on solution in capsules; and as a result 25 percent of them regenerated. The early stages were filamentous and similar to the regeneration from the *Aspidium marginale* from Long Island; but in this experiment the heart-shaped prothalli were slow to develop and a large number of the filamentous structures died without producing them at all. This difference in regeneration in the two cultures may be due to some difference in constitution or to differences in external conditions resulting from the different seasons. The results of experiments made to determine whether regeneration in *Aspidium marginale* varied with the seasons were inconclusive.

The production of sporophytic buds on leaves cut off to induce regeneration was as rare in this culture as in the preceding one.

No chromosome counts were made in the regenerated prothalli, but there was no indication that they differed from the $2x$ prothalli of *Aspidium marginale* in which chromosome counts were made, and in all probability the chromosome number here is the $2x$ number also. The prothalli were similar to the ones in which counts were made and bore normal sex organs which produced sporophytes. From the studies on cell size described above, it is evident that the increase in length of the cells of the lower

epidermis of the leaf is the most marked characteristic of a young tetraploid plant of this species. Consequently, a study was made of the length of the lower epidermal cells in both the tetraploid and diploid plants of this culture, the stage of development of the embryo as a whole being the same as in similar studies made in the preceding culture.

When these measurements were compared with those made on *Aspidium marginale* from Long Island, it was found that the differences were not significant, being not more than twice their probable errors.

TABLE 3. *Cells of Leaves of Aspidium marginale (Pennsylvania)*

Sporophyte	No. Cells Measured	Lower Epidermis Lengths in μ	Difference	% Increase
$2x$	200	119.90 ± 1.62		
$4x$	200	225.11 ± 3.02	105.21 ± 3.42	88

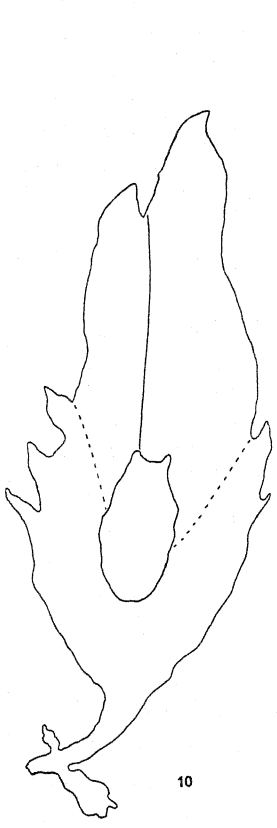
Sporophytic Structures on Prothalli

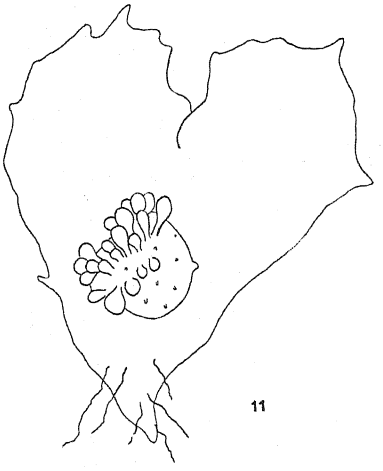
A few abnormal plants developed, showing combinations of gametophytic and sporophytic characteristics. Text figure 11 shows a regenerated gametophyte which was heart-shaped but without a cushion and it consisted of a single layer of cells except for the mass of tissue which bore sporangia. Glandular hairs, such as are found on young leaves of sporophytes, were present on the tissue bearing the sporangia. Sections of the thickened region showed that it contained a few tracheids. The sporangia themselves (text fig. 12) were borne on stalks which were shorter and thicker than the typical stalks of the Polypodiaceae. Development ceased and the sporangia became a lighter green without forming mature spores and the annulus was never conspicuously differentiated. Sections showed a sporangial wall of a single layer of cells and the remains of what may have been a tapetum. The sporogenous tissue was in the sixteen cell stage in all the larger sporangia and the nuclei were in a resting condition. Since in no case more than sixteen cells were found, it appeared that the sporangia developed much like normal sporangia up to meiosis but at that point the nuclei became somewhat abnormal in appearance, and none of the sporangia showed any signs of the reduction division.

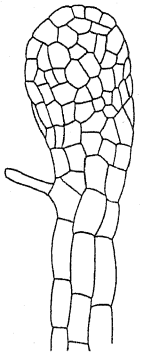
Another $2x$ prothallus developed a cushion and bore sporangia on one side of the prothallus and archegonia on the other side. A sporophyte developed from one of the archegonia.

Text figure 10 illustrates a plant whose posterior part was distinctly prothallial in character and consisted of a single layer of cells. The anterior part (in front of the dotted lines) resembled a leaf, having a mid-rib with tracheids, upper and lower epidermis, and a well developed mesophyll extending about half way from the mid-rib to the margin of the leaf. The mean length of these epidermal cells was found to be 113.70 ± 2.20 microns. The difference between the length determined for normal sporophytes and

this figure is only 2.33 times its probable error. Therefore these cells resemble the corresponding cells of the normal sporophytes, which might be expected since the chromosome number is in all probability $2x$ in each case. The mass of cells at the place where the gametophyte tissue meets







TEXT FIG. 10. *Aspidium marginale.* Plant regenerated from leaf of diploid sporophyte. The posterior part was prothallus-like and the part in front of the dotted lines was leaf like. It contained a mid-rib, epidermal cells, and stomata.

TEXT FIG. 11. *Aspidium marginale.* Prothallus regenerated from leaf of diploid sporophyte. Sporangia were borne on the thickened region.

TEXT FIG. 12. *Aspidium marginale.* Single sporangium from prothallus shown in figure 11.

the sporophyte tissue contained tracheids and when it first appeared seemed to be an early stage in the development of an apogamous sporophyte, but it never formed a leaf. Rhizoids were present both on this thickened structure and on the prothallial tissue.

Summary of Results of Experiments with Aspidium marginale

Regeneration from leaves of normal sporophytes gave gametophytes from which sporophytes were derived sexually. Chromosome counts were made in the normal and regenerated gametophytes, in the normal sporophytes, and in those produced on regenerated gametophytes. The chromosome numbers were doubled, giving $2x$ gametophytes and tetraploid sporophytes. The leaves of the $4x$ sporophytes regenerated prothalli bearing both kinds of sex organs. Probably the chromosome number in these gametophytes was the $4x$ number; but counts have not been made.

The cells of the regenerated prothalli, both the $2x$ and the $4x$, were increased in size, particularly in width. The cells of the $4x$ sporophytes were larger than those of the $2x$. Due to the great increase in length, but not in width, of the lower epidermal cells of the leaf, those cells were of a different shape from the corresponding cells of the $2x$ plant. A few regenerated prothalli were found bearing sporangia.

Woodwardia virginica (L.) Sm.

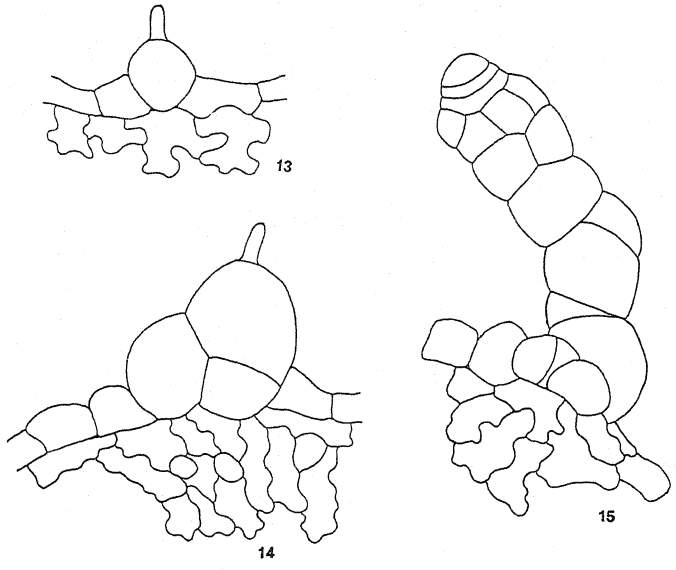
Spores of *Woodwardia virginica* were collected near Lake Ronkonkoma, Long Island, New York, and were germinated on Shive's nutrient solution. Regenerated prothalli appeared in about four weeks after the leaves were cut off and cultured. It was evident at once that it was particularly easy to induce regeneration in this fern, since 60 percent of the 50 leaves cut regenerated. Although the original plants were all grown on Shive's solution, both Shive's and Knopf's solutions were used for regeneration, and they seemed to be equally good.

The Plants Obtained by Regeneration

The regenerated prothalli developed from single cells, either on the margins or on the surfaces of the leaves. Text figures 13–15 show the development of a filamentous prothallus from a single cell. A mass of filamentous prothalli covered with antheridia developed on the regenerating leaves first, and from the filamentous gametophytes heart-shaped ones were formed with well developed cushions bearing archegonia. The cultures grown from spores contained a large number of small male gametophytes at first; the bisexual plants developed a little later. Therefore the regenerated prothalli were following the normal course of development for this fern. The antheridia were normal and the sperms were motile. There were 32 or 64 sperms in each antheridium as in the normal plants.

Sporophytes developed very soon after the first archegonia appeared on the regenerated prothalli. Experiments to prevent fertilization were also made with this fern. Twelve gametophytes with mature sex organs were kept on agar for two months. Sporophytes did develop on three of these prothalli because antheridia were so numerous; and the prothalli seemed to

grow in a horizontal position instead of standing somewhat on edge as in many ferns. Therefore fertilization could take place very easily if a drop of water fell from the lid of the Petri dish. The remaining nine plants never produced sporophytes. The number of plants used in this experiment to prevent fertilization was small due to lack of material. Observations showed that the sporophytes formed on the regenerated prothalli of *Woodwardia* were not apogamous, but that they grew from the archegonia.



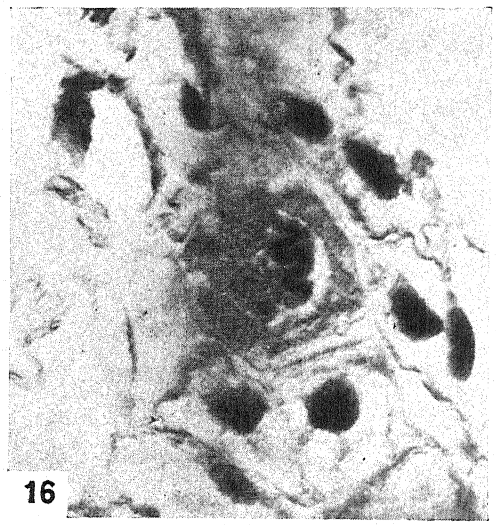
TEXT FIGS. 13–15. *Woodwardia virginica.* Three stages in the development of a filamentous prothallus from a single cell on the margin of a leaf.

Conclusive evidence of fertilization in the *2x* prothalli of *Woodwardia* was obtained. Some of the prothalli bearing mature sex organs, which had been kept on agar for two months without the formation of sporophytes, were placed in a small glass capsule and covered with nutrient solution for about three hours. At the end of that time they were fixed in formol-acetic-alcohol, embedded in paraffin, and cut. A section was obtained showing sperms in the open neck of an archegonium and a single spirally coiled sperm within the nucleus of the egg (text fig. 16).

Root Regeneration

A number of roots regenerated and produced the same kinds of structures as the leaves. The green roots were cut off and cultivated for about ten weeks before the filamentous prothalli appeared. A little chlorophyll was present in the tips of the roots before they were cut off; but after they were detached, the tips became much greener and chlorophyll was developed for some distance back from the tip. This was in contrast to the roots of

Woodsia obtusa which were green only near the tips. The filamentous prothalli grew from cells on the surface or very near the surface of the root. The *2x* gametophytes produced only antheridia in the filamentous stage, but later heart-shaped prothalli with archegonia grew from the filamentous ones. Single regenerating roots were isolated and grown on sand and solu-



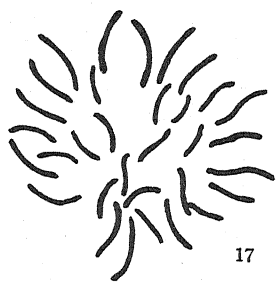
TEXT FIG. 16. *Woodwardia virginica.* Fertilization in a diploid prothallus.

tion until sporophytes developed. These sporophytes were similar to the ones produced on the prothalli regenerated from leaves. Regenerated tissue from the roots of *Woodwardia* was always in the form of prothalli bearing sex organs; while the regenerated tissue formed on the roots of *Woodsia* was either filamentous prothalli without sex organs or sporophytic buds. In each species in which root regeneration occurred, the results were exactly like the regeneration from the leaves of that species.

Chromosome Numbers in Woodwardia

The chromosome numbers show also that fertilization took place before the development of the embryos. Counts were made in normal prothalli, regenerated prothalli, normal sporophytes, and sporophytes developed from regenerated gametophytes, with the results given in table 4. The number of chromosomes is large and therefore cannot be determined exactly from somatic tissue, but reduction stages were not available. In each tissue in which the chromosomes were counted, a number of careful counts were made from different cells. Mitotic figures were easier to obtain in the gametophytes of *Woodwardia* than in *Aspidium* because so many antheridia

were produced on small prothalli. The most accurate counts were made in the first division of the central cell which gives rise to the sperms, because in later divisions the chromosomes of two cells might be confused. The chromosomes of the x gametophyte and of the $2x$ gametophyte are shown in text figures 17 and 18.



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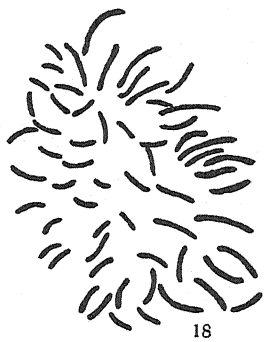


18

TEXT FIG. 17. *Woodwardia virginica.* Chromosomes at metaphase from cell of haploid gametophyte.

TEXT FIG. 18. *Woodwardia virginica.* Chromosomes at metaphase from cell of diploid gametophyte.

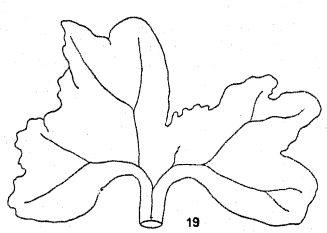
TABLE 4. *Chromosome Numbers in Woodwardia virginica*

Normal prothalli	32 ±
Regenerated prothalli	57– 64
Normal sporophytes	59– 65
Sporophytes on regenerated prothalli	123–130

Comparison of the Regenerated Plants with Normal Plants

The leaves of the tetraploid plants were much more irregular in outline and often showed foldings and thickenings, as well as lobes which were peculiar in shape and not fully developed. Text figures 19 and 20 show such differences between leaves of young tetraploid and diploid plants.

The results of the microscopic study of the sizes of the cells are shown in table 5. The areas were obtained in the manner described for *Aspidium*.



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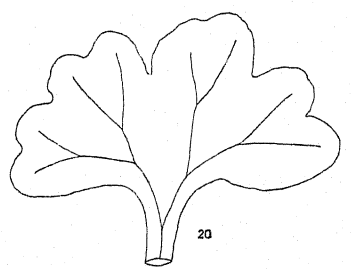

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TEXT FIG. 19. *Woodwardia virginica.* Leaf of young tetraploid sporophyte.

TEXT FIG. 20. *Woodwardia virginica.* Leaf of young diploid sporophyte.

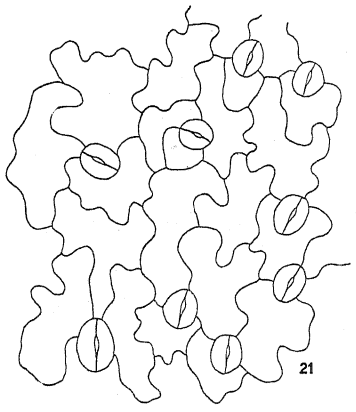
That is, the areas of the prothallial cells are the products of the two dimensions. The areas of the epidermal cells were determined by measuring with a planimeter. Ten cells from the tetraploid plant and the same number from the diploid plant were measured from camera lucida drawings. From these measurements the actual areas as shown in table 5 were calculated. Cells were chosen whose dimensions were consistent with the dimensions shown in the table. Text figures 21 and 22 show the shape of lower epidermal cells of the diploid and tetraploid plants.

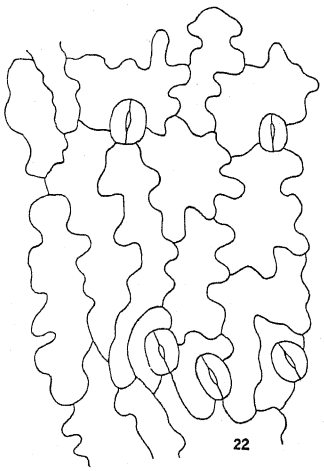
TABLE 5. *Cells of Woodwardia virginica*

Prothallus	No. Cells Measured	Lengths in μ	Difference	% Increase
x	300	114.07 ± 1.15		
$2x$	300	135.51 ± 1.47	21.44 ± 1.86	19
		Widths in μ		
x	300	55.79 ± .42		
$2x$	300	72.44 ± .54	16.65 ± .68	30
		Areas in sq. μ		
x		6400		
$2x$		9800		53
Sporophyte	Lower Epidermal Cells of Primary Leaves			
	No. Cells Measured	Lengths in μ	Difference	
$2x$	300	117.43 ± .97		
$4x$	300	175.27 ± 1.70	57.84 ± 1.96	49
		Widths in μ		
$2x$	300	65.45 ± .45		
$4x$	300	85.05 ± .76	19.60 ± .89	30
		Areas in sq. μ		
$2x$		3646		
$4x$		6252		71

A comparison of the results of the measurements of the prothallial cells of *Aspidium* and *Woodwardia* showed that there was a greater increase in area in *Woodwardia*. As in *Aspidium* the length of the $2x$ cells did not show as great a change as the width. In neither *Aspidium* nor *Woodwardia* were the areas of the $2x$ cells two or three times the areas of normal cells, a condition reported by Heilbronn (11) for *Polypodium aureum*. The area of the lower epidermal cells of *Woodwardia* was enlarged 71 percent as compared with 75 percent in *Aspidium* and 120 percent in *Polypodium*.

A few leaves of the tetraploid sporophytes regenerated to form gametophytes which probably have the $4x$ chromosome number. Both archegonia and antheridia have been produced on these prothalli.





TEXT FIG. 21. *Woodwardia virginica.* Lower epidermal cells of diploid sporophyte.
TEXT FIG. 22. *Woodwardia virginica.* Lower epidermal cells of tetraploid sporophyte.

Attempts to Produce Triploid Sporophytes

In *Woodwardia* the production of small male gametophytes and large bisexual gametophytes with few antheridia made possible an experiment to produce triploid sporophytes. The regions bearing antheridia were cut off some of the large $2x$ prothalli which had been growing on agar for some time. These prothalli, each with many mature archegonia, were put in solution in a small glass capsule together with male gametophytes having the x chromosome number. After a few hours the large $2x$ prothalli were removed and further cultivated. At the end of several weeks each of the prothalli had developed at least one sporophyte. Theoretically the resulting sporophytes should have the $3x$ chromosome number. Due to the manner in which the sporophytes were obtained, it is impossible to be sure that they are triploids until chromosome counts can be made. The possibility of an antheridium on the $2x$ prothalli being overlooked makes the results uncertain, although a careful search was made for antheridia before the $2x$ prothalli were put in the capsule. The sporophytes are quite young and no measurements have been made on the cells of the leaves. The method described here for the production of triploid sporophytes can be applied to any fern which produces prothalli of this type; that is, prothalli bearing antheridia in regions where they can be easily found and removed. It is believed that by further experiments along this line triploid sporophytes can be developed in several species.

Summary of Results of Experiments with Woodwardia virginica

Regenerated prothalli of the typical heart-shaped form were produced on young leaves and young roots of diploid sporophytes. The cells of the

regenerated prothalli were larger than those of normal prothalli and the chromosome number was double that of prothalli grown from spores. Sections of $2x$ prothalli showed the sperm within the egg nucleus. Sporophytes developed from the sex organs of the $2x$ gametophytes. The leaves of the resulting sporophytes differed in appearance and were composed of larger cells than those of normal plants. The chromosome number was the $4x$ number. The leaves of the $4x$ plants also regenerated to produce prothalli bearing archegonia and antheridia.

Discussion

From the large number of Filicales and Bryophytes which have responded to experiments on induced apospory, it is evident that it is not a phenomenon peculiar to a few species. Previous to the present work regeneration had been induced in the leaves of twelve species of ferns belonging to four different families. By the experiments described in this report regeneration was obtained in eleven species, ten of which are here reported for the first time. Regeneration in *Osmunda regalis* was also obtained by Goebel (9).

Species Regenerated

The following species showed regeneration:

Pteris cretica var. *albo-lineata* Hook.
Woodwardia virginica (L.) Sm.
Asplenium platyneuron (L.) Oakes
Athyrium filix femina var.
Scolopendrium vulgare Sm.
Polystichum acrostichoides (Michx.) Schott
Aspidium marginale (L.) Sw.
Cystopteris fragilis (L.) Bernh.
Woodsia obtusa (Spreng.) Torr.
Dicksonia punctilobula (Michx.) Gray
Osmunda regalis L.

Regenerated structures were not obtained from the following:

Pteris aquilina L.
Camptosorus rhizophyllus (L.) Link.
Aspidium spinulosum var. *intermedium* (Muhl.) D. C. Eaton
Onoclea sensibilis L.
Osmunda Claytoniana L.

The primary and secondary leaves of the embryo and in some species the third leaves and the primary roots were capable of growth. Only a few attempts were made to regenerate prothalli from older leaves and they were unsuccessful. However, Heilbronn (11) has reported regeneration from mature leaves of *Polypodium aureum*.

Growth from the leaves was from epidermal cells on either the margin

or the surface and from the petiole. The prothalli produced on the roots probably came from superficial cells also, but on roots the origin is more difficult to determine. The sporophytic buds on the roots of *Woodsia* grew from cells beneath the root cap.

Factors Influencing Regeneration

Although it was found to be very easy to induce regeneration in many ferns, very little was learned about the cause or conditions necessary for regeneration to take place. Since it is known that external conditions influence to a marked degree the growth and development of prothalli arising from spores, it is to be expected that the environment plays an important role in the formation of prothalli from detached leaves. Two different nutrient solutions, Shive's and Knopf's, as well as different concentrations of these solutions, were used for some ferns. Leaves were cut not only at all seasons of the year but for *Woodsia obtusa* every month of the year. Experiments were made in three different laboratories: at the University of Michigan, at Hunter College, and at the Biological Laboratory at Cold Spring Harbor, where there were some variations both in temperature and in the amount of light. Not all of the ferns were tried in all three laboratories but most of them were grown and regenerated in at least two places.

Two collections of spores from plants growing in different localities were made for *Aspidium marginale* and *Asplenium platyneuron*. It was not found that variations in any of the environmental factors which have been mentioned had any definite influence on regeneration. *Woodsia* was tried on different solutions, different concentrations of the solutions, different times of the year, and in different laboratories with the same results. Filamentous prothalli and sporophytic buds were formed but never heart-shaped prothalli. *Aspidium marginale*, whether from spores collected at the Botanical Gardens of the University of Michigan or from Cold Spring Harbor, formed heart-shaped prothalli at all seasons and in both the laboratories where it was tried.

Therefore regeneration is not peculiar to particular plants of a species and it can take place on different concentrations of different nutrient solutions. However, it is necessary to cut the leaves before they are so old that they are beginning to lose their bright green color. Cultures contaminated with algae did not usually regenerate or if growth did begin, the prothalli lived only a short time. Therefore young sporophytes which had been grown in pure culture gave the best results, and cultures on solution could be kept uncontaminated more easily than those on agar or sphagnum.

Structures Produced by Regenerating Leaves

The structures produced by the regenerating leaves were of three distinct types, although some species produced only two types. A few

sporophytes were formed by budding on leaves of *Aspidium marginale* and *Asplenium platyneuron;* and the buds were very common on the leaves and roots of *Woodsia obtusa*. The sporophytes produced by these buds resembled normal plants and the chromosome number was in all probability the diploid number.

Filamentous prothalli were produced as one of the first results of growth in the regenerating leaves in nearly all the species studied. In the early stages they were comparable to the filaments produced by germinating spores but they persisted for a longer time. They bore rhizoids and occasionally antheridia. The filamentous stage was persistent in *Woodsia* and the $4x$ prothalli of *Aspidium marginale* remained filamentous for nearly six months instead of becoming heart shaped in a few weeks as the $2x$ prothalli did.

Heart-shaped prothalli developed from the filamentous prothalli of *Aspidium marginale*, *Cystopteris fragilis*, *Woodwardia virginica*, *Osmunda regalis*, *Athyrium filix-femina* var., *Pteris cretica* var. *albo-lineata*, *Asplenium platyneuron*, and *Polystichum acrostichoides*. *Pteris* produced no sex organs and *Asplenium* and *Polystichum* only antheridia. All the others developed both kinds of sex organs.

Polyploidy in the Gametophyte Generation

In two species, *Aspidium marginale* and *Woodwardia virginica*, gametophytes with the $2x$ chromosome numbers were produced. The evidence for the diploid character of these gametophytes depends on chromosome counts made in the prothalli grown from spores and in the regenerated prothalli. Since the regenerated gametophytes in all the species were produced in a similar manner, that is, by regeneration from diploid leaves, it is believed that diploid gametophytes were obtained in eleven species of ferns. Similarly, gametophytes produced by regeneration from tetraploid sporophytes are believed to have the $4x$ chromosome number. Such gametophytes were produced in two species, *Aspidium marginale* and *Woodwardia virginica*. Fern gametophytes with the $4x$ chromosome number have not previously been produced.

The regenerated heart-shaped prothalli resembled the normal ones except that they were composed of larger cells in all the species in which cell measurements were taken. In every species the width of the cells showed a greater increase than the length, a fact also observed for *Polypodium aureum* by Heilbronn (11). Heilbronn states that the area of the normal prothallial cells was to the area of the regenerated as 1 : 2 and often as 1 : 3 or 1 : 4. In no species studied by the writer was so great a difference found. The area of the cells of the regenerated prothalli of *Aspidium* showed an increase of 34 percent; those of *Woodwardia*, 53 percent. A comparison of these results with the tables of cell sizes for regenerated and normal gametophytes of mosses given by Wettstein (24)

showed that for some species of mosses the increase was no greater than for the species of ferns reported here.

Polyploidy in the Sporophyte Generation

It is known that tetraploid plants were produced in two species, *Aspidium marginale* and *Woodwardia virginica*. Chromosome counts were made in the normal plants and in the plants formed on regenerated prothalli of both species and fertilization was observed in the $2x$ gametophytes of *Woodwardia*. Therefore there can be no doubt but that the sporophytes have the $4x$ chromosome number.

The tetraploid plants of *Aspidium* and *Woodwardia* differed from the diploid in the same manner. The irregularities in the leaves were more pronounced in *Woodwardia*. The cells of the primary leaves were much larger in both species. The area of the lower epidermal cells of *Aspidium* was 75 percent greater, and those of *Woodwardia* 71 percent greater, than the corresponding cells of the normal plants. Heilbronn (11) found a 120 percent increase for *Polypodium aureum*. No other fern sporophytes with the $4x$ chromosome number have been produced experimentally. The difference in size in the epidermal cells of *Aspidium* was most pronounced in the length, while in *Woodwardia* the length and width were almost equally enlarged, giving cells of approximately the same shape in the tetraploid as in the diploid.

The measurements made on normal mature plants of *Aspidium* showed no significant difference in size when compared with the measurements made on the primary leaves of the same species. Therefore the size of the cells in the primary leaf indicates the size in the leaves of the mature plants and it is to be expected that the mature tetraploid plants will be composed of cells which are larger than those of the diploid plants.

Several years are required to produce mature sporophytes. Therefore all tetraploid plants are being carefully cultivated in order to bring them to the fruiting stage. The events occurring at meiosis will prove interesting and a study will be made of spore formation when material is available.

Apogamy and Aposporv

A discussion of apogamy and aposporv as they occur in nature has not been attempted in this paper. Aposporv induced by removing and cultivating leaves of young sporophytes is the result of unusual cultural conditions and at present offers no explanation for aposporv as it occurs spontaneously. The production of sporangia, epidermal cells, and stomata on regenerated prothalli occurred only on a small percentage of the aposporous gametophytes.

Lang (12, 14) has reported the appearance of sporangia on prothalli of two species of ferns which were apogamous if fertilization was prevented until the gametophytes became very large. The chromosome numbers

have not been determined in these ferns. The situation in *Aspidium*, which also produced sporangia on the prothalli in these cultures, differs in that the prothalli were not old and fertilization had not been prevented. The prothalli studied by Lang were grown from spores, while those of *Aspidium* were produced by regeneration, and had the diploid chromosome number. A sporophyte was produced from an archegonium on the same prothallus which bore sporangia.

Induced apospory ordinarily does not change the sequence of events in the life cycle. This was found to be true for *Polypodium aureum* by Heilbronn (11) and by Wettstein for the mosses (24). Aposporous prothalli of *Aspidium* and *Woodwardia* which were derived from sexually produced sporophytes reproduced sexually; and the aposporous prothalli with the $4x$ chromosome number were not apogamous. On the other hand, *Pteris cretica* var. *albo-lineata* was always apogamous and the regenerated prothallus from that species was apogamous.

Summary

1. Apospory was induced in eleven species of ferns.

2. The aposporous gametophytes of five species were heart-shaped, and bore both archegonia and antheridia. The cells of the aposporous gametophytes were larger than the cells of gametophytes grown from spores.

3. Some ferns produced sporophytic buds and filamentous prothalli on the detached leaves. The sporophytes originating from the buds resembled normal plants.

4. The aposporous prothalli of *Aspidium marginale* and *Woodwardia virginica* produced sporophytes from the sex organs. Fertilization was observed in *Woodwardia*. Chromosome counts showed that these plants were tetraploids.

5. The leaves of the tetraploid sporophytes were abnormal in appearance and were composed of larger cells than the leaves of the diploid sporophytes of the same species.

6. Apogamy does not necessarily follow induced apospory.

7. *Pteris cretica* var. *albo-lineata* was normally apogamous and a regenerated prothallus of this fern was also apogamous.

8. Some of the regenerated prothalli of *Aspidium marginale* bore sporangia and some contained stomata, epidermal cells, and tracheids together with prothallial cells.

9. Sporophytes of *Woodwardia virginica* which probably have the triploid chromosome number were produced by crossing $2x$ plants with x plants.

10. Gametophytes which were believed to have the $4x$ chromosome number were produced by regeneration from the leaves of *Aspidium* and *Woodwardia*. The $4x$ prothalli produced both archegonia and antheridia.

This work was completed in the Botanical Laboratory of the University of Michigan under the direction of Dr. Carl D. LaRue who suggested the problem and to whom the writer wishes to express her sincere appreciation for assistance during the course of the investigation.

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THE FULL-CYCLE PUCCINIAS ON ONAGRACEAE IN NORTH AMERICA[1]

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During an examination of the collections of *Puccinia Epilobii-tetragoni* (DC.) Wint. on Onagraceae in the Arthur Herbarium it became evident that the assignment of synonyms and hosts in the North American Flora could scarcely represent the facts. The grouping was originally presented by Bisby (1916). The present paper is an attempt to delimit, chiefly on morphologic grounds, the many North American forms and is essentially the scheme adopted by Holway (1907). There is considerable divergence from the species conception of Bisby, and of Arthur (1920).

The abundant material now available makes possible a more exact assignment of hosts. In some cases the descriptions of telia and teliospores are emended to correspond to facts derived from microscopic studies.

THE AGGREGATE SPECIES P. EPILOBII-TETRAGONI

Puccinia Epilobii-tetragoni as treated by Bisby includes all of the autoecious full-cycle forms of the genus *Puccinia* known to occur on onagraceous hosts in North America. His list of synonyms contains thirty names and the species is given as utilizing eleven host genera and many species. Sydow (1904) recognized, in the same group, nine species and Holway five. The present writer by considering *Puccinia heterantha* Ellis & Ev. as synonymous with *P. Oenotherae* Vize accepts as valid morphologic species the following: *P. Oenotherae* Vize, *P. glabella* Holw., *P. Gayophyti* Billings, and *P. Epilobii-tetragoni* (DC.) Wint.

MORPHOLOGY OF THE SORI AND SPORE FORMS

Pycnia are of small systematic use in this group of species. They occur throughout, most abundantly in *P. Oenotherae*, especially on *Taraxia* (*P. heterantha*), variable but usually few in *P. Epilobii-tetragoni*, and doubtfully in *P. Gayophyti*. Pycnia and aecia are unknown in *P. glabella*.

It seems fairly certain that the known collections of aecia on *Epilobium* having abundant pycnia belong to the heteroecious species, *Puccinia Veratri* Duby.

Aecia are likewise of little help but like the pycnia are useful in separating *Epilobium* collections. The aecia of *P. Epilobii-tetragoni*, aside from being

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

accompanied by few pycnia, are smaller than those of *P. Veratri* and appear in early spring rather than in summer.

The urediniospores are so nearly alike as to yield no satisfactory basis for determinations; in all species they are obovoid, globoid, or often oblate-spheroid, and measure 14–19 by 19–26 μ. The pores are two and always approximately equatorial, the wall variable but between 2 and 3 μ, the marking and color quite constant throughout. If emphasis is placed on urediniospores or aeciospores there is justification for such a species grouping as urged by Bisby.

If the emphasis given the teliospore is the same as that accorded the urediniospore in this group, the decision will surely be different. The type of telium, the teliospore size, wall marking, apical thickening, germ-pore location, and type of pedicel provide definite and easily observable characters for the onagraceous Puccinias. Lindroth (1902) in his study of the rusts on Umbelliferae concluded that the teliospores were of first importance in properly showing relationships in that group of rusts and pointed out that little use had been made of the pore location.

The species descriptions as given by Holway are generally accurate. The teliospore wall markings are not correctly given in *P. Gayophyti*, which he describes as smooth. A careful examination of many collections reveals that this is rarely if ever true. The wall is minutely rugose or more often noticeably reticulate, most readily seen in ten percent sodium hydroxide solution, a mounting medium which has been found to be very satisfactory for examination of teliospores with fine wall markings. Examination should follow immediately after mounting, as the wall swells quite rapidly and soon obscures its markings.

Brief discussions of each of the four species follow. On account of their uniformity, as previously stated, the pycnia, aecia, and uredinia are not included in the discussion of the species.

1. Puccinia Oenotherae Vize

Grev. 5: 109. 1877

Puccinia Boisduvaliae Peck, Bot. Gaz. 7: 45. 1882.

Puccinia Clarkiae Peck, Bull. Torrey Club 11: 49. 1884.

Puccinia heterantha Ellis & Ev., Erythea 1: 204. 1893.

Puccinia Eulobi Diet. & Holw., Erythea 1: 249. 1893.

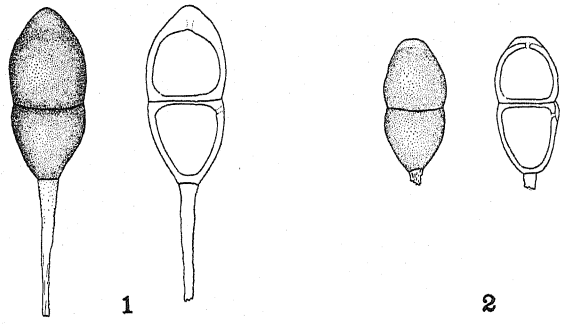
Puccinia Sphaerostigmatis Diet. & Neg., Bot. Jahrb. Engler. 22: 353. 1896.

Puccinia Zauschneriae Syd., Monogr. Ured. 1: 435. 1903.

Telia amphigenous, sometimes caulicolous, pulvinate, compact or rarely pulverulent, chocolate or blackish-brown; teliospores (text fig. 1) ellipsoid or oblong-clavate, 16–24 by 27–55 μ, rounded or rarely acute above, rounded below, slightly constricted at septum; wall 1–3 μ thick at sides, thickened 3–13 μ above, chestnut-brown, smooth; pore of upper cell usually apical,

of lower cell at septum; pedicel hyaline or slightly tinted, usually long and persistent but sometimes short and largely fragile.

HOSTS: *Boisduvalia densiflora* (Lindl.) S. Wats., Calif., Wash.—*B. parviflora* A. Heller, Ida.—*B. salicina* (Nutt.) Rydb., Ida., Ore., Wash.—*B. sparsiflora* A. Heller, Calif.—*B. stricta* (Gray) Greene, Ore.—*Chylisma cardiophylla* (Torr.) Small, Calif.—*C. hirta* A. Nels., Nev.—*Clarkia amoena* (Lehm.) Nels. & Macbr., Calif., Ore.—*C. biloba* (Durand) Nels. & Macbr.,



TEXT FIG. 1. Teliospores of *Puccinia Oenotherae* showing the smooth, chestnut-brown spore-wall, much thickened at the apex. Germ pores in both cells are apical and the pedicel is typically long and persistent as shown. × 625.

TEXT FIG. 2. These smooth-walled, cinnamon-brown teliospores are characteristic of *Puccinia glabella*. The apex is slightly thickened by a differentiated umbo, the pedicel is short and fragile and the pore of the lower cell is at or near the septum. × 625.

Calif.—*C. elegans* Dougl., Calif.—*C. epilobioides* (Nutt.) Nels. & Macbr., Nev., Wash.—*C. pulchella* Pursh, Ida., Ore., Wash.—*C. rhomboidea* Dougl., Calif., Wash.—*C. superba* Nels. & Macbr., Calif.—*C. sp.*, Calif.—*Epilobium minutum* Lindl., Calif., B. C.—*Eulobus californicus* Nutt., Calif.—*Oenothera strigosa* (Rydb.) Mackenzie & Bush, Mont., Wash.—*Sphaerostigma alyssoides* (H. & A.) Walp., Calif., Ida.—*S. andinum* (Nutt.) Walp., Ida., Wash.—*S. bistoria* (Nutt.) Walp., Calif.—*S. Boothii* (Dougl.) Walp., Ore., Wash.—*S. contortum* (Dougl.) Walp., Calif., Wash.—*S. decorticans* (H. & A.) Small, Calif.—*S. dentatum* (Cav.) Walp., Ore.—*S. hirtellum* (Greene) Small, Calif.—*S. micranthum* (Hornem.) Walp., Calif.—*S. pubens* (S. Wats.) Rydb., Calif.—*S. spirale* (Lehm.) Walp., Calif.—*S. utahensis* Small, Utah —*S. Veitchianum* (Hook.) Small, Calif.—*S. viridescens* (Lehm.) Walp., Calif.—*S. sp.*, Ariz., Calif.—*Taraxia breviflora* (T. & G.) Nutt., Mont.—*T. graciliflora* (H. & A.) Raim., Calif.—*T. longiflora* Nutt., Nev.—*T. ovata* (Nutt.) Small, Calif.—*T. subacaulis* (Pursh) Rydb., Colo., Ida., Mont., Nev., Utah, Wyo.—*T. sp.*, Nev.—*Zauschneria californica* Presl., Calif.—*Z. Garrettii* A. Nels., Utah.

The inclusion here of *P. heterantha* on *Taraxia* is not in accord with Holway's treatment. It is a smooth-walled form with the general size range and wall color of the other forms of *P. Oenotherae*, but the teliospores

are slightly shorter and broader, 19–26 by 30–45 μ. It often differs from collections on other hosts in having an apex usually only slightly thickened and a fragile pedicel. In addition, the upper pore often lies below the thickened apex. Pycnia are more abundantly formed than on other hosts. The present writer considers it best to include it with *P. Oenotherae*.

2. Puccinia glabella Holw.

N. Am. Ured. 1: 76. 1907

Telia amphigenous, at first covered by the gray epidermis, early pulverulent, dark cinnamon-brown; teliospores (text fig. 2) oblong, 14–19 by 24–34 μ, usually rounded above and below, slightly constricted at septum; wall 1–2 μ thick at sides, thickened 2–3.5 μ above by a semi-hyaline, differentiated umbo, smooth, cinnamon-brown; pore of the upper cell apical, of lower cell at or near septum; pedicel hyaline, very short and fragile.

Hosts: *Boisduvalia glabella* (Nutt.) Walp., Ore., Nev.

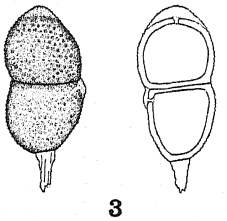
This rust when originally described was considered by Holway as "a very different thing" from *P. Boisduvaliae* Peck (*P. Oenotherae*, as here considered). Bisby admits that Holway was justified in his view but still places it with his *P. Epilobii-tetragoni*. It is known on only the one host, which it shares with *P. Epilobii-tetragoni* proper. The teliospores of *P. glabella* are smooth-walled but of lighter wall color, more fragile pedicel, and considerably smaller size than *P. Oenotherae*. The apex is distinctly umbonate, not merely thickened. In general size and morphology of the telia and teliospores *P. glabella* approaches the next two species to be considered but differs from both in having teliospores with a smooth wall and from *P. Epilobii-teiragoni*, in addition, in the location of the germ pore of the lower teliospore cell.

3. Puccinia Gayophyti Billings

U. S. Geol. Expl. 40th Par. 5: 414. 1871

Puccinia Gayophyti Peck, Bot. Gaz. 7: 56. 1882.

Puccinia Gayophyti Speg., Anal. Mus. Nac. B. Aires III 1: 63. 1902.



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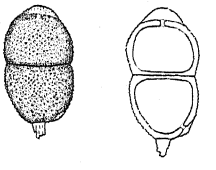

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Text Fig. 3. The teliospores of *Puccinia Gayophyti* are dark cinnamon-brown with short fragile pedicels. The wall is obscurely rugose or reticulate with a differentiated umbo over the pore of the upper cell at the apex and over the lower pore at the septum. × 625.

Text Fig. 4. Teliospores of *Puccinia Epilobii-tetragoni* showing the dark cinnamon-brown, verrucose wall. The wall is thickened over the apical pore and over the pore of the lower cell near the pedicel by a differentiated umbo. The pedicel is short and fragile. × 625.

Telia amphigenous, scattered but often confluent over the entire leaf surface, chocolate-brown; teliospores (text fig. 3) ellipsoid, 16–23 by 27–40 μ, rounded above and below, slightly constricted at septum; wall 1.5–3 μ thick at sides, thickened 3–6 μ above by a semi-hyaline, differentiated umbo, finely rugose or noticeably reticulate, dark cinnamon-brown; pore in upper cell apical, in lower cell at septum; pedicel short, hyaline, fragile.

HOSTS: *Gayophytum caesium* T. & G., Ida., Nev., Utah, Wash., Wyo.—*G. diffusum* T. & G., Calif., Ida., Utah.—*G. Helleri* Rydb., Ida.—*G. lasiospermum* Greene, Utah.—*G. Nuttallii* T. & G., Ida.—*G. pumilum* S. Wats., Calif., Ida.—*G. racemosum* T. & G., Ariz., Colo., Ida., Wyo.—*G. ramosissimum* T. & G., Calif., Colo., Ida., Mont., N. M., Ore., Utah, Wyo.—*G. sp.*, Calif., Colo., Ore., Utah, Wyo.

In the original description no note is made as to teliospore-wall marking, but the teliospore wall has generally been considered to be smooth. Bisby, however, found the wall often to be verrucose, while the writer considers it to be most often reticulate, and only rarely is it smooth.

In size the teliospores approach *P. glabella* (16–23 by 27–40 μ) and are smaller than *P. Oenotherae*. The rugose-reticulate wall, however, is sufficient to delimit *P. Gayophyti* from *P. glabella*. The pore of the lower cell is always at the septum, a character which is sufficient to distinguish *P. Gayophyti* from *P. Epilobii-tetragoni* proper.

4. PUCCINIA EPILOBII-TETRAGONI (DC.) Wint.

Rabenh.-Wint. Krypt. Fl. 1: 214. 1881

Uredo vagans α Epilobii tetragoni DC., Fl. Fr. 2: 228. 1805.
Puccinia pulverulenta Grev., Fl. Edin. 432. 1824.
Puccinia intermedia Diet. & Holw. Bot. Gaz. 18: 254. 1893.

Telia amphigenous, early pulverulent, often confluent, chocolate-brown; teliospores (text fig. 4) ellipsoid or somewhat oblong, 14–21 by 26–38 μ, rounded above and usually below, slightly constricted at septum; wall 1.5–2.5 μ thick at sides, thickened 3–7 μ above by a semi-hyaline differentiated umbo, dark cinnamon- or chestnut-brown, finely rugose or verrucose-reticulate, often appearing striolate; pore of upper cell apical; pore, or two pores, of lower cell midway to the pedicel or below; pedicel hyaline, short, fragile.

HOSTS: *Boisduvalia glabella* (Nutt.) Walp., Ida.—*B. stricta* (Gray) Greene, Ore.—*Chylisma scapoidea seorsa* A. Nels., Ida.—*Epilobium adenocaulon* Hausskn., Mont., N. M., Wash.—*E. americanum* Hausskn., Ida., Wash.—*E. francescanum* Barbey, Calif.—*E. jucundum* A. Gray, Wash.—*E. minutum* Lindl., Ore.—*E. paniculatum* Nutt., Calif., Colo., Ida., Mont., Nev., N. D., Ore., S. D., Utah, Wash., Wyo.—*E. sp.*, Colo., Ore., Wash., Wyo., Sask.—*Gayophytum diffusum* T. & G., Ida.—*G. racemosum* T. & G., Ariz., Colo.—*G. ramosissimum* T. & G., Wash.—*G. sp.*, Ariz., Colo.

As the description indicates, this species is similar to *P. Gayophyti* but differs in the position, and quite often the number, of the germ pores of the

lower teliospore cell, as pointed out by Holway. Bisby agrees that this distinction holds in general but did not consider it as absolutely constant. The writer has found that it is an entirely dependable character.

The list of hosts assigned to this species differs from those of previous writers in that while it occurs largely on *Epilobium* it is also considered to occur sparingly upon *Boisduvalia glabella*, *Chylisma scapoidea seorsa*, and three species of *Gayophytum*. This overlapping of hosts undoubtedly influenced Bisby in his opinion that these rusts are too intergrading to be worthy of specific rank.

SUMMARY

1. *Puccinia Epilobii-tetragoni*, as treated in the North American Flora, is divided into four species, *P. Oenotherae* Vize, *P. glabella* Holw., *P. Gayophyti* Billings and *P. Epilobii-tetragoni* (DC.) Wint.

2. *Puccinia Oenotherae* has teliospores measuring 16–24 by 27–55 μ. The teliospore-wall is chestnut-brown, smooth and thickened 3–13 μ above. The germ pore of the upper cell is apical, of the lower cell at the septum. The pedicel is long and usually persistent.

3. The teliospores of *P. glabella* measure 14–19 by 24–34 μ. The wall is cinnamon-brown, smooth and thickened 2–3.5 μ at the apex by a differentiated umbo. The germ pore of the upper cell is apical, of the lower at or near the septum. The pedicel is short and fragile.

4. The teliospores of *P. Gayophyti* measure 16–23 by 27–40 μ. The wall is dark cinnamon-brown, finely rugose or reticulate and thickened 3–6 μ at the apex by a differentiated umbo. The germ pore of the upper cell is apical, of the lower at the septum. The pedicel is short and fragile.

5. The teliospores of *P. Epilobii-tetragoni* are similar in size and wall characters to those of *P. Gayophyti* but the pore of the lower cell is near the pedicel, which is short and fragile.

6. Host and localities are given for each of the species.

The writer wishes to thank Dr. J. C. Arthur for many helpful suggestions.

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CHROMOSOME BEHAVIOR IN TRIPLOID *PETUNIA* HYBRIDS[1]

WILLIAM CAMPBELL STEERE

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INTRODUCTION

During the summer of 1928, several species and a large number of horticultural varieties of *Petunia* were grown at the Botanical Gardens of the University of Michigan, for cytological and genetical investigation. The seed was obtained from various commercial sources and through the kindness of a number of Botanical Gardens and individuals.

The large flowered forms commonly known as "California Giants," "Diener's Monsters," etc., were of especial interest, since they appeared to be typical *gigas* forms. Because of the unusually large and fleshy flowers and leaves, combined with the large stature of the plant, they were assumed to be tetraploids, since Vilmorin and Simonet (54) had reported that such types exist in the genus. Later in the season, the tetraploid nature of the *gigas* types was established.

With the possibility in mind of producing triploids, reciprocal crosses were made between tetraploid and diploid types, but very little seed was produced, and none germinated.

During the summer of 1929, the reciprocal crosses were repeated between diploids and tetraploids on a larger scale than the previous year. A pure strain of *P. axillaris* (Lam.) BSP. (*P. nyctaginiflora* Juss.), which has white flowers and yellow pollen, was used as the diploid parent (Pl. XXIII, fig. 2). As parthenogenesis has occasionally been reported in the Solanaceae, strains of tetraploids with dark colored flowers and blue pollen were chosen as the other parent. From these crosses, large amounts of seed were secured, especially from the cross diploid ♀ × tetraploid ♂.

The F_1 plants of the reciprocal crosses were grown and examined during the summer of 1930. Unexpectedly large progenies were secured from the diploid ♀ × tetraploid ♂ cross, a total of 1348 plants resulting from the contents of about fifteen seed capsules. In each of two cultures there were nearly 500 plants, and the remaining cultures contained somewhat smaller numbers. Approximately half of these plants were intermediate in size between the two parents, while the rest resembled the diploid plant almost exactly in stature and habit. The flower color of each parent was represented in the progeny, as well as intermediate shades.

It was not believed likely that so many triploids could be obtained, and subsequent examination of random plants has shown that apparently they

[1] Papers from the Department of Botany, University of Michigan, number 365.

are normal diploids. They cannot have arisen parthenogenetically, but must have contained a certain number of chromosomes contributed by the pollen parent, as the resemblance in habit and flower color showed. Different proportions of each set of parental chromosomes were apparently present in these F_1 plants, since there was such a wide range of variation, especially as one of the parents (*P. axillaris*) is known to be a pure strain. The cytology and genetics of this cross will be reported later.

The Triploid Material

The F_1 generation from *tetraploid* ♀ × *diploid* ♂ cross was quite different in appearance and chromosomal constitution from the progeny of the reciprocal, previously described. Out of a large amount of seed, only 16 plants appeared, belonging to two different cultures. By examination of the pollen mother cells of these plants in aceto-carmin they were all found to be triploids.

One of the cultures consisted of eleven plants which were the progeny of a tetraploid with deep red flowers and blue pollen, pollinated by *P. axillaris*. This group, hereafter designated as culture 264, showed great variation among the plants, not only in color and size of flowers, but in growth habit and stature as well.

According to their appearance, these individuals fitted rather clearly into three classes. Five plants (2, 4, 5, 8, 11) resembled the tetraploid in their large fleshy habit, although the flowers were not so massive. The flowers were of the same shape as those of the diploid parent, three being of the same color (white), and two were of a deep red-purple color (Pl. XXIII, fig. 5).

Four plants (3, 6, 9, 10), all very similar in appearance, seemed to show more of the paternal characters than the preceding class. The habit of the plants and the size and shape of the flowers were all very similar to those of the pollen parent, but the flowers, instead of being white, were reddish-purple with blue pollen (Pl. XXIII, fig. 4).

The last class consisted of two individuals (1, 7) of an unexpected and unexplained character. Instead of being intermediate between the two parents in size and general appearance, these plants were almost exactly similar to the pollen parent, but very noticeably smaller, otherwise differing only in having blue pollen (Pl. XXIII, fig. 3).

The other triploid culture consisted of five plants and was secured by pollinating a pink-flowered tetraploid (Pl. XXIII, fig. 1) with pollen from *P. axillaris*. This group of plants will be referred to subsequently as culture 266. There was less variation among the individuals of this group than in the preceding one, and the plants resembled the tetraploid parent in size and general habit more than they did the diploid. One plant (4) was especially close to the tetraploid type in appearance, except that the flowers were somewhat smaller and light purple in color (Pl. XXIII, fig. 6).

The remaining plants resembled *P. axillaris* in flower shape, but were considerably larger and more fleshy in all respects, and had blue pollen. Two plants (3, 5) had purple-pink flowers, whereas two had the white flowers characteristic of the diploid parent.

In addition to the sixteen triploid plants just described, the author was kindly permitted to study a group of nine triploid individuals in the cultures of Dr. E. E. Dale, who had also made the *tetraploid* × *diploid* crosses. These nine plants were also examined cytologically and found to be characteristic triploids. The whole group of twenty-five different triploids supplied abundant cytological material, upon which this paper is based.

All of the triploid plants were found to be self-fertile, and also to set seed when pollinated by either parent. Most triploids have been reported to be completely self-sterile, and sterility has come to be considered typical of them.

Cuttings made from each triploid plant are maintained at the University of Michigan Botanical Garden. Back crosses and progenies secured by self-pollination are being investigated.

Methods

For counts of somatic chromosomes, root tips and styles were fixed in Taylor's modification of Flemming's chrom-osmo-acetic mixture (51), which was found to be superior to several acetic-alcohol fixatives tried at the same time. Most of the cytological studies were of microsporogenesis. The anthers were fixed as follows: Several terminal branches bearing flower buds were removed and kept fresh by wrapping in moist towelling. One anther from each flower bud was examined in aceto-carmin, and if satisfactory stages were found, the remaining anthers were fixed. Thus much labor was saved, since nearly all the fixed material exhibited the desired stages of meiosis. Immediately after being placed in the fixing solution the anthers were cut into small pieces with a sharp scalpel in order to facilitate rapid penetration.

The root tips, styles, and anthers were then dehydrated, cleared, and imbedded in paraffin. The series of mixtures of butyl and ethyl alcohols for dehydrating and clearing suggested by Zirkle (56) was tried and found to be perfectly satisfactory. Sections were cut sufficiently thick to include entire cells, and stained by Newton's gentian-violet and iodine method (39).

For permanent records of chromosome counts, smears were made. Many were fixed in Taylor's modification of Flemming's solution, and stained in gentian violet. Others were fixed and stained in aceto-carmin, dehydrated in glacial acetic acid and absolute alcohol mixtures, cleared in an equal mixture of xylene and absolute alcohol, and mounted in balsam. This method, as previously described (Steere, 50), is rapid and quite satisfactory for chromosome counts.

Cytological Results

So far as known, the basic haploid number in the genus *Petunia* is seven, which has been found in all the pure species yet examined. This number has been reported for *P. violacea* Lind. (Ferguson, 9, 10; Skalinska and Cuchtman, 48; Malinowski, 34; Vilmorin and Simonet, 52; and others), *P. axillaris* (Lam.) BSP. (*P. nyctaginiflora* Juss.) (Ferguson, 10), and *P. Parodii* Steere (Steere, 49). *P. inflata* Fries, which was also examined, showed seven pairs of chromosomes during the reduction division.

On account of its almost complete self-sterility, the true *P. violacea* is extremely hard to keep under cultivation, so that very few living specimens of the pure species are now in existence outside of their natural range.

The commercial *P. "violacea,"* which includes the common garden types, has been derived from crosses among *P. violacea*, *P. axillaris*, and *P. inflata*, so that it is a composite of two or three species, certain characteristics of each appearing in varying degrees in different strains. A horticultural name, *P. hybrida*, has been applied with considerable aptness.

Seeds of *P. "violacea"* secured from various botanical gardens gave strains of *P. hybrida*, *P. inflata*, or other species. The author has not as yet been able to secure a satisfactory pure strain of *P. violacea*, even after a continued search. It is therefore strongly suspected that many of the studies of nominal *"P. violacea"* have actually concerned either a closely related species or a hybrid. The latter alternative is the more probable for the reports of irregular meiosis, such as non-reduction, the production of microcytes, and similar phenomena, since these are characteristic manifestations of hybridity. The reports of loose pairing or no pairing of the chromosomes also suggest a strongly heterozygous or hybrid condition. From descriptions of the material, *P. hybrida* can frequently be recognized as having been used.

Of the three pure species in the author's cultures, *P. axillaris*, *P. inflata*, and *P. parodii*, the first was selected for the production of triploids because of the very regular type of meiosis, the large size of plants and flowers, and the high degree of self-fertility. This species has also been kept in culture longer and was known to be relatively pure genetically.

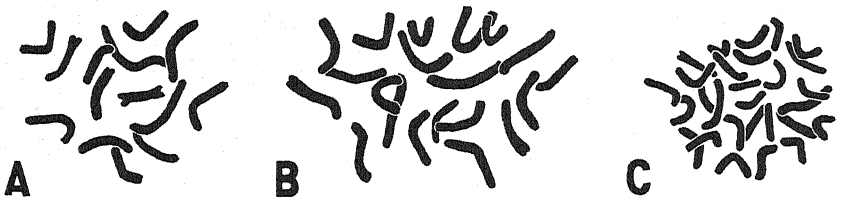
Cytology of the Diploid P. axillaris

The somatic divisions in the root tips of *P. axillaris* are very regular, the 14 chromosomes being easily counted at metaphase (text fig. 1, *A*). No great size differences among the chromosomes are evident, and no constrictions, satellites or other distinctive morphological characters were found.

Skalinska and Cuchtman (48) have reported that certain polymorphic races of *P. "violacea"* show great variability in the size and shape of the somatic chromosomes. They further found that satellite-like bodies are sometimes present in the tissues of zygomorphic flowers, and are lacking in

normal flowers on the same plant. Malinowski (34) has investigated a similar race of *P. "violacea"* which has both large and small variegated flowers on the same plant, and reports that large chromosomes are found in the large flowers, and small ones in the small flowers. He concludes that "variegation is connected in *Petunia* with the size of the chromosomes."

These remarkable findings were not observed in *P. axillaris*, in which the behavior of the somatic chromosomes is very regular.



TEXT FIG. 1. Somatic chromosomes from root-tip cells. *A*, *Petunia axillaris* ($2n = 14$); *B*, Triploid form of *P. hybrida* ($2n = 21$); *C*, Tetraploid *P. hybrida* ($2n = 28$).

The regularity characteristic of mitosis continues throughout meiosis in *P. axillaris*. At diakinesis the chromosomes are closely paired, some side by side, others end to end, into seven gemini. The loose pairing ascribed to *P. "violacea"* by various workers was not observed in *P. axillaris*. The first division is very regular, and no instances of non-disjunction or unequal distribution of chromosomes were seen. The homotypic division is also regular and no abnormalities were seen in the large number of pollen mother cells examined. The tetrads are normal and without microcytes, and produce very few sterile pollen grains.

Matsuda (35) describes and figures a number of anomalous cytological conditions in *P. "violacea,"* the most noteworthy of which are (1) the gemini at diakinesis are often joined together, sometimes into a complete ring, (2) in the early homotypic division the chromosomes may again become arranged in a ring, (3) during the homotypic division a darkly staining body "believed to be siderophile matter" was seen between the two spindles. These phenomena are undoubtedly caused by slow or faulty fixation. The anthers of *Petunia* species are protected by a heavy layer of cutin, which prevents the fixative from penetrating rapidly, so that the locules must be cut open in order to secure good fixation. The author has observed the same conditions described by Matsuda in anther locules which were not cut into during fixation, and into which the fixative penetrated very slowly. The remaining irregularities reported, as loose pairing of chromosomes, with the consequent appearance of univalents, lagging chromosomes, unequal distribution of chromosomes in either division, the formation of restitution nuclei, suppression of one or both divisions, with the production of monads, diads, triads, etc., are all believed to be due to the hybrid condition of the material.

Cytology of the Tetraploid P. hybrida

The strains of *gigas*-type petunias which are sold under various trade names, such as "California Giants," "Diener's Monsters," etc., arose spontaneously over 40 years ago. According to Bailey (1) they have been derived from one plant which appeared suddenly in the cultures of Mrs. Thomas Gould at Ventura, California, in 1888. Although this was probably not the first tetraploid mutation in *Petunia*, it was at least the first which received attention and which was cultivated as a new type.

Two other tetraploid mutants have appeared very recently. Kostoff (25) reports the spontaneous appearance of a larger plant in a culture of diploid plants. The mutational form blossomed over a week earlier than the other plants, and showed other physiological and morphological differences. Cytological examination of the root-tips showed 28 diploid chromosomes. The rediction division was found to be very irregular, and only about 50 percent of the pollen proved to be fertile. Furthermore, the plant was entirely self-sterile. When pollen from a diploid plant was used, however, seed was obtained. Among the progeny of this cross appeared sixteen plants which also were found to be tetraploids but which differed considerably from the maternal parent in appearance, and produced only about two percent of sterile pollen (Kostoff and Kendall, 26). The meiotic process was much less irregular in the F_1 tetraploid progeny, the chromosome complex having been stabilized in some way. Other aberrant types also appeared among the progeny of this cross.

Dermen (7) also reports the sudden appearance of a tetraploid *Petunia* in a diploid population. It had very large flowers and leaves, but when self-pollinated, it gave both large-flowered and small-flowered tetraploids, showing that it was heterozygous for flower size.

Gigantism is often regarded as a consequence of the doubling of the chromatic material, but there is a possibility that concomitantly with doubling of the chromosomes there may sometimes be mutations of factors affecting size. The author has in his cultures tetraploids which are extremely large and fleshy as well as some which can be distinguished from diploids only with difficulty. On the other hand, diploid forms exist which almost exactly resemble tetraploids, and are hard to distinguish from them without careful examination. The large size of tetraploids is believed to be in part independent of the chromosome doubling, and so is a genetic rather than a physiologic expression.

Among the commercial tetraploid strains, a great deal of variation in cytological behavior was noticed. A few strains showed considerable aberration, the whole meiotic process being disturbed, much as in the tetraploid mutation described by Kostoff. In general, however, sporogenesis in the tetraploids is entirely regular, as in the diploid *P. axillaris*. This is to be expected, for although the tetraploid arose from a hybrid, the doubling of the chromosome sets provided a duplicate or homologue

for each member of the set, so that complete pairing was possible. The same phenomenon was observed in hybrids between *Brassica* and *Raphanus* by Karpetschenko (21), who found that even though meiosis in the original hybrid was very irregular, with complete lack of pairing of the chromosomes, unequal segregation, etc., the same process in tetraploid hybrids was quite regular, since the two homologous sets from each parent were present, and paired *inter se*.

Cytology of the Triploid Hybrids

The somatic chromosomes were counted in the root tips and styles of 16 triploid specimens, and each plant was found to have the expected number of 21. The mitotic divisions are very regular, and no abnormalities were observed in the distribution of the chromosomes. As in the other forms studied, no striking size or morphological differences were visible among them. The dissimilarity of appearance of root tip chromosomes and those found in the somatic tissues of the young flowers is worthy of note. The root tip chromosomes are longer, more slender, and show more definite differences in size and shape. The chromosomes in the meristematic tissue of the flower are much condensed, and show very little difference in size among them. (Compare text figure 1 with figure 1 of Plate XXIV.)

Meiosis was found to be very similar in all the individuals. The meiotic process is surprisingly regular, considering the unbalanced chromosome complement. A detailed study of the prophases was not made, although some stages were examined. Paired threads were sometimes seen, but three parallel threads were never observed.

At early diakinesis the chromosomes are regularly united into sets of three (Pl. XXIV, fig. 2). Although one or two of these trivalent chromosome complexes may break up during late diakinesis, there are usually seven trivalents at the heterotypic metaphase (Pl. XXIV, figs. 3–6). This normal organization of the total chromosome complement into trivalents is very unusual, and has hitherto been reported in very few plants. In the majority of triploids so far known, only a partial production of trivalent chromosomes has been reported. In only a few instances have triploids without any trivalents at all been reported, notably in hybrid forms, which show only bivalents and univalents at diakinesis.

Dermen (7) has recently reported upon triploidy in *Petunia*, but since two hybrid forms were used as parents, the cytological behavior of his triploids was much less regular. For example, he did not find a complete association of all the chromosomes into trivalents.

In the author's triploid petunias, the chromosomes making up the trivalents are united in different ways, straight chains of three being the most common union, showing that several sets of three are closely homologous. Commonly, the third member is attached at one point of union of a pair. Sometimes the third member is attached to both members of a separated pair, as though the terminal chromosomes of a chain of three

had started to pair together. These and other variations are shown in Plate XXIV, figures 3–5. Apparently there is not much constancy as to the proportion of each kind of trivalent in different microsporocytes. This inconstancy of arrangement of the chromosomes into trivalents is well illustrated by a trisomic mutation under investigation in which the extra chromosome is found united in a number of ways with its two homologues.

Two chromosomes from each trivalent normally pass to one pole, and the third to the opposite pole (Pl. XXIV, figs. 7–8). Distribution of single and paired chromosomes is entirely at random, so that the expected segregation of ten and eleven chromosomes generally occurs. No great size differences are apparent among the constituent members of any trivalent, so that the paternal and maternal chromosomes are apparently of the same size. Thus the chromosomes of the *gigas* or tetraploid type are concluded to have just as much chromatin as those of the diploid. The individual chromosomes are almost spherical or elliptical, and all of approximately the same size. No distinctive morphological characters are visible, although, as mentioned before, there is some variation in the type of connection between the three chromosomes in each set.

Counts are difficult during the first division, since the chromosomes are crowded together, and further, it is often difficult to distinguish between bivalents and univalents.

When the nuclear walls appear around the disc-shaped telophasic chromosome group, the individual chromosomes do not undergo a complete reorganization and form a spireme, but remain separate and distinct throughout the interkinetic period. They become gradually distributed throughout the new nuclear cavities, which grow larger and change in shape from hollow discs into hollow spheres (Pl. XXIV, figs. 12–13, Pl. XXV, figs. 1–2). The chromosomes parallel these changes in nuclear size and shape by very characteristic alterations in their appearance. The occasional pairs of chromosomes which arrived at the poles still intact, now disjoin, and the component parts become entirely distinct. The nuclei at this stage are very large and nearly fill the sporocyte (Pl. XXV, fig. 1). That the interkinetic phase persists for a relatively long period is manifested by the large number of preparations which show this stage.

The individual chromosomes begin to exhibit signs of the coming homotypic division by a characteristic constriction at the center, and a change in shape. They assume, gradually, a dumb-bell form, or more rarely the form of a short-armed cross (Pl. XXV, figs. 1–2). The large spherical nuclei begin to contract, crowding the chromosomes together again, but into a spherical group (fig. 3). This early prophase may easily be distinguished from the preceding telophase by the spherical nucleus and the peculiarly constricted chromosomes. The nuclear walls disappear and leave the homotypic chromosomes in two closely-packed groups on different sides of the cell (fig. 4). The spindles form rapidly and the chromosomes

become oriented upon them (figs. 4–7). The two spindles may be parallel or at right angles to each other, or at an intermediate angle. The metaphase plates are very even and regular, with the exception of a few abnormalities which will be discussed later. The already partly split chromosomes complete the division and the two halves of each one pass very regularly to opposite poles (figs. 8–10).

The telophasic chromosomes in well fixed material are always separate and distinct, and may easily be counted. New nuclear walls appear and the chromosomes become evenly distributed throughout the inclosed cavities (Pl. XXV, fig. 11). They become smaller and somewhat indistinct, as the nuclear cavity enlarges. Separation of the cells of the tetrad is due entirely to progressive furrowing of the cytoplasm, as no visible cell plates are laid down during either division (fig. 12). By the time cleavage is completed and the cells have become entirely separated within the wall of the old pollen mother cell, the chromosomes have anastomosed and formed a nuclear reticulum. The chromatin becomes more and more indistinct, as the nuclear cavity increases in size, and by the time the pollen grains are mature, the nucleus is in a resting condition and very hard to stain satisfactorily.

Good preparations of the first somatic mitosis of the microspore were not secured, due to the large amount of stored food, and the sensitiveness of the cells to changes in osmotic pressure. This division was found to occur before the pollen grains are shed, however.

The phases through which the nucleolar material passes were not followed through meiosis and subsequent stages, because its affinity for gentian violet apparently disappears about the middle of prophase and does not reappear until the pollen grains are mature.

Irregularities of the Triploid Plants

Since the *Petunia* triploids are cytologically and genetically unbalanced, their meiotic stages show a certain amount of irregularity, but not so much as that reported for other triploids. There is less irregularity in the writer's than in Dermen's triploid petunias.

At diakinesis one or more of the trivalents may break up into a bivalent and a univalent, or into three univalents. Any separation at all is of rare enough occurrence so that it must be considered as abnormal. The univalents migrate irregularly to the poles, often lagging behind or preceding the main group of chromosomes. The bivalents may migrate entire to one pole, or the members may segregate regularly. During the anaphase of the heterotypic division, lagging chromosomes frequently appear (Pl. XXV, figs. 10–11). The most common cause of this type of irregularity is the passing to the poles of the terminal members of a chain of three chromosomes, leaving the central chromosome at the equator of the spindle. Such lagging chromosomes may become completely isolated and cut off in

the cytoplasm, or they may finally migrate slowly to one pole or the other and become included in one of the daughter nuclei. They often divide prematurely, the half chromosomes going to the same or to different nuclei, or remaining on the spindle (figs. 10–11). Occasionally a bivalent chromosome is seen to lag (fig. 11). In addition to disjoining, the constituent chromosomes frequently split and may either be segregated regularly or remain in a group as the nucleus of a small cell. During interkinesis, many of the chromosomes split prematurely, prior to the homotypic division. These divided chromosomes are easily recognized in late interkinesis by their spherical form and small size, and by the usual proximity of both halves (fig. 2).

Because of this early splitting, the sum of the chromosomes present in the second division metaphase plates is frequently more than 21, and may be as high as 26 (Pl. XXV, fig. 6). On the theoretical basis of chance distribution, there should be just as many plates of eleven chromosomes as plates of ten. On account of lagging and other irregularities causing chromosome loss, there should be a reduction in the number of plates of eleven, and an increase in plates of ten. It was observed, however, that plates of eleven are far in the majority, in spite of the chromosome loss, and plates of twelve are just as common as plates of ten, which presents a considerable deviation from the expected segregation (table 1). Careful examination indicated that distribution of chromosomes in the first division is entirely according to the laws of probability, and the discrepancy in numbers of chromosomes in the second division is accounted for by precocious splitting of lagging and interkinetic chromosomes.

TABLE 1. *Counts of Metaphase II Plates in Triploid Petunias*

Number of chromosomes	6	7	8	9	10	11	12	13	14
Number of plates	2	8	40	68	104	144	104	22	10

There are not as many irregularities during the homotypic division as in the heterotypic. The halves of the chromosomes which split prematurely may lag and disintegrate, or become small, separate nuclei (Pl. XXV, fig. 10). The chromosomes cut off in the cytoplasm during the first division continue their activity, dividing and forming small independent nuclei. These microcytes produced by isolated chromosomes and groups of chromosomes are common in the triploid material and are directly proportional in size to the number of chromosomes which they contain (fig. 13).

None of the major irregularities reported by other writers appeared, such as complete non-reduction, the formation of restitution nuclei, the union of the interkinetic nuclei, or the fusion of the two homotypic spindles. A very few giant pollen grains, apparently containing all 21 chromosomes, were seen, but the abnormal processes leading to their production were not observed. The meiotic processes in these particular triploid petunias are even more regular than meiosis in certain types of diploid *P. hybrida*, such as the one reported by Matsuda.

Discussion and Conclusions

The term triploid has been used in a narrow sense by some workers, and in a broad sense by others. Belling (2) has defined a triploid plant, in the narrow sense, as one having a chromosome complex made up of a number of sets of three homologous chromosomes. According to this interpretation, there must be three identical groups of chromosomes present in each triploid cell. It is to be expected, on the basis of a similar behavior in diploids, that at the reduction division, all of the chromosomes will become arranged into groups of three. This expectation is not usually realized, however, as such an association of all the chromosomes into trivalents has been reported for very few plants.

Belling has reported complete trivalency in *Datura* (5), *Canna* (2, 4) and *Hyacinthus* (3). The same condition is described in the present paper in *Petunia*. It has also been described in two wild Japanese species of *Lycoris*, *L. radiata* Herb. and *L. squamigera* Maxim., by Inariyama (18). All of these constant triploids or "autotriploids" display a certain amount of meiotic irregularity which often results in the fragmentation of some of the trivalent chromosomes into univalents and bivalents. In addition to these slight irregularities of the autotriploids, triploids in closely related varieties and forms of *Canna* (Tokugawa and Kuwada, 53), *Hyacinthus* (Darlington, 6), and *Petunia* (Dermen, 7) do not show complete trivalency. Apparently, then, the complete association of homologous chromosomes in triploids is rarely possible, and is the unusual thing. Darlington found, in *Hyacinthus* (6), that homology is not the only factor in the formation of trivalents, but that size also is important, since short chromosomes form trivalents much less frequently than long ones do.

By far the majority of the triploids which have yet been investigated have been found to diverge to a greater or less extent from the expected complete association into sets of three. Although most triploids display trivalent chromosomes at late diakinesis and at early metaphase of the heterotypic division, the proportion of trivalents may often be very small, as compared with the number of bivalent and univalent chromosomes. This erratic and variable production of trivalents is representative of nearly all triploids which have been reported.

The greatest possible deviation from the expected or autotriploid type is shown by those plants which form no trivalent chromosomes at all, but have only bivalent and univalent chromosomes. Most of the instances reported have been hybrids which acquired two sets of chromosomes from one parent and one set from the other, the two parents belonging to different species or even genera. Under such circumstances, there can be very little association of the chromosomes into trivalents, since they are only slightly or not at all homologous. The earliest and best known examples of this type were the *Drosera* hybrids secured by crossing *D. rotundifolia* ($n = 10$) with *D. longifolia* ($n = 20$) (Rosenberg, 45). Rosenberg found no trivalents

at all, but ten pairs and ten unpaired chromosomes. Similar behavior has been reported since in species hybrids of *Rosa* (Erlanson, 8), and *Triticum* (Kihara, 23; Sax, 47; Thompson, 52). Two sets, probably from one of the parents, pair off together, leaving the remaining set from the other parent as isolated univalents, which behave independently of the bivalents.

The application of the term triploid to these plants is believed to be fully justified, in spite of the differences in association and arrangement of their chromosomes, since there is a high degree of resemblance among them. They all possess an unbalanced chromosome complex, consisting of three sets of chromosomes, and consequently exhibit many meiotic irregularities, which result in the production of a large amount of sterile pollen. Further, they are usually sterile and somewhat larger than the normal diploid type.

It is believed, then, that the innate character of the triploid does not depend on whether or not the chromosomes are homologous, but simply on the fact that there are three sets present. Whereas, in general, the presence of trivalents is considered one of the distinguishing features of triploid plants, it cannot be taken as the sole criterion. It is concluded that our definition of a triploid plant should be made broader and more general, so that the term may include any plant which possesses three times the basic number of chromosomes characteristic of the genus, without regard to the degree of homology among the three sets as indicated by the number of trivalents.

Since there is such a gradual transition between complete association of all the chromosomes into trivalents, and the complete absence of chromosome aggregation, it is hardly possible to classify triploids on the basis of chromosome behavior, as is usually done. In an attempt to find some other means of classifying them, it was discovered than nearly all triploids can appropriately be placed in one of two groups, on the basis of maintenance of the triploid character.

In the first group belong all the triploid species and varieties which are able to maintain their unstable chromosome complex from generation to generation. Since irregular segregation and extinction of the type would naturally follow sexual reproduction, and since most triploids are sterile, multiplication must of necessity be by some asexual means, which is sometimes rather obscure.

Certain species and varieties belonging to this first class of triploids are able to maintain their triploid nature and consequent taxonomic individuality by various types of apomixis and the resulting production of seed from which new triploid individuals will arise. Rosenberg (46) found that a constant wild species of *Hieracium*, *H. umbellatum*, is triploid, and that the embryos in the seed which produce triploids arise from nucellar buds. Holmgren (17) found similar phenomena in various naturally occurring species of *Eupatorium* and *Erigeron*. Most members of this group preserve their triple set of chromosomes by more obvious means of vegetative

reproduction. Typical triploid species and varieties of this sort have been found in a large number of genera which multiply by bulbs, stolons, rhizomes, etc. They have been described in *Iris* (Kazao, 22), *Rubus* (Longley, 28), *Rosa* (Erlanson, 8), *Tulipa* (Newton and Darlington, 40), *Hyacinthus* (Belling, 3, 6), *Narcissus* (Nagao, 36), *Canna* (Belling, 2, 4; Tokugawa and Kuwada, 53), *Hemerocallis* (Belling, 4), *Fragaria* (Yarnell, 55), and others. In general, these triploids have appeared spontaneously, either in the natural state, or under cultivation, and are probably the result of natural or artificial hybridization.

The second and larger group of triploids is composed of the forms or races of preëxisting species which are not able to retain cytological and systematic individuality from generation to generation, since they do not habitually reproduce vegetatively. Some members of this group can be artificially propagated, however. Although a few examples are unstable wild triploid species, as those found in the genus *Crataegus* (Longley, 29), and in *Prunus* (Okabe, 41), most known triploids have arisen spontaneously as chromosome mutations among cultivated or experimental plants. Thus, *Oenothera semi-gigas* has appeared several times, and has been found to be characterized by 21 somatic chromosomes (Lutz, 32). Triploid mutations of *Datura* (Belling, 5), *Zea* (McClintock, 33; Randolph and McClintock, 44), *Crepis* (Navashin, 37), *Nicotiana* (Goodspeed, 16), tomato (Lesley, 27), *Morus* (Osawa, 43), and *Primula* (Ono, 42) have all arisen among normal diploid plants.

Other interesting triploids are those which have been produced experimentally as the result of crossing a tetraploid and a diploid race of the same species, as was done in the present investigation. *Oenothera* (Gates, 12; Geerts, 15) and *Solanum* (Jørgensen, 20) triploids have been produced in the same way. The crossing of two species, either in the same or in different genera has also given rise to triploid hybrids, as reported for *Triticum* (Kihara, 23, 24; Sax, 47; Thompson, 52), *Drosera* (Rosenberg, 45), *Citrus* × *Fortunella* (Longley, 31), and *Zea* × *Euchlaena* (Longley, 30).

These triploids of known origin are important, since through them desirable genetic characters may be interchanged between varieties or species with different chromosome numbers. Navaschin (37) found that crosses which normally were invariably unsuccessful would give fertile seed, if a triploid was used as the female parent. He found also, that by self-pollinating and crossing triploids, he was able to get higher polyploid forms, of still greater cytological and genetical interest. Also, considerable light is shed upon the origin of spontaneous triploid mutations by this type of triploid.

The sudden appearance of triploids among diploids has been the source of much speculation for two decades. Gates (14) has advanced the theory that triploid mutations are due to the union of two sperm nuclei with a single haploid egg. The only recorded observations that might be inter-

preted to support this hypothesis are those of Nemec (38) on *Gagea lutea* and Ishikawa (19) on *Oenothera*. That there is not much probability of such a phenomenon is evident, and the experimental evidence is slight. Concerning the observations of Nemec, Gates (13) himself says: "Nemec believes that he has observed this (dispermy) in *Gagea lutea*, although it must be said that the evidence is not very conclusive." Ishikawa, however, observed and figured what was apparently the fusion of two sperm nuclei with a single egg nucleus.

Probably the best experimental evidence against Gates's theory has been produced by Navaschin (37), who found that triploids which arose spontaneously through the open pollination of *Crepis capillaris* contained a diploid *capillaris* complex, in addition to a foreign set of chromosomes. It is manifest that dispermy cannot have occurred here, but that an alternative theory is necessary. Navaschin's triploids have been produced through the normal fusion of two gametes, one haploid and the other diploid, the latter being the egg. It is very evident that triploids produced experimentally by crossing diploid and tetraploid races, as, for example, the writer's triploid Petunias, certainly might have arisen only by the fusion of a diploid and a haploid gamete.

That gametes with the somatic number of chromosomes are often produced has long been known. Hybrid plants, as described earlier, are frequently characterized by irregular meiosis, and produce, as a result, a certain number of diploid and other non-typical gametes. Under the stimulation of extremes of temperature or other abnormal physiological conditions, nearly any plant may be induced to produce diploid or polyploid gametes. The production of such gametes has been reported in a large number of species. By subjecting buds from the parent plant, as well as seeds and seedlings, to X-rays, Goodspeed (16) produced both triploid and tetraploid plants in *Nicotiana tabacum*. The spontaneous appearance of polyploid types in culture has frequently been correlated with unfavorable physiological conditions which were responsible for the production of irregular gametes.

Many attempts have been made to produce triploids by pollinating diploids with pollen from tetraploids. In the few instances in which the cross was successful, diploid plants have almost invariably resulted, as the author found. Apparently the only exception is that of Dermen (7), who secured triploids by the diploid ♀ × tetraploid ♂ cross. Except for this one instance, all of the induced triploids have resulted from the cross tetraploid ♀ × diploid ♂. It is probable, then, that whereas a diploid sperm nucleus may rarely function, the diploid chromosome set of a triploid is almost always derived from the egg. Some objections have been raised concerning the possibility of fertilization of a diploid egg. Navaschin has shown, however, that in *Crepis* diploid eggs are capable of being fertilized as well as haploid ones, and that even higher polyploid egg cells are perfectly

functional. Triploid, tetraploid, pentaploid, and heptaploid plants were all found in the immediate progeny of triploids.

The majority of triploids, then, and probably all which have arisen spontaneously, owe their existence to the fertilization of a diploid egg by a haploid sperm nucleus.

Summary

1. The tetraploid nature of the giant-flowered races of *Petunia hybrida* has been confirmed.

2. Reciprocal crosses were made between a tetraploid race of *P. hybrida* ($n = 14$) with colored flowers and a diploid species, *P. axillaris* ($n = 7$), which has white flowers and yellow pollen.

3. The diploid ♀ × tetraploid ♂ cross produced normal diploids which combined characters from both parents.

4. Sixteen triploid plants resulted from the tetraploid ♀ × diploid ♂ cross, each with 21 somatic chromosomes.

5. The triploid progeny were about intermediate between the two parents in size and habit, although two, of similar appearance, were noticeably smaller than either parent.

6. At the reduction division, the triploids normally show a complete association of all the chromosomes into sets of three.

7. The first and second divisions of meiosis are very regular, the only common irregularity being lagging of chromosomes. None of the extreme irregularity described for other triploids was seen.

8. A characteristic idiosyncrasy is the premature splitting of chromosomes during interkinesis. As a result, there is frequently an apparent excess of chromosomes at the homotypic division.

9. A considerable part of the pollen in the triploids is sterile, partly due to microcytes arising from chromosomes which lagged or became isolated during meiosis, and partly to cells which do not contain a complete or functional set of chromosomes.

10. A classification of triploids on the basis of maintenance of the triploid character rather than upon the degree of chromosome association into trivalents is suggested.

11. It is concluded, from the results of the present investigation and from others, that triploids, with very few exceptions, have arisen from the union of a diploid egg with a haploid sperm nucleus.

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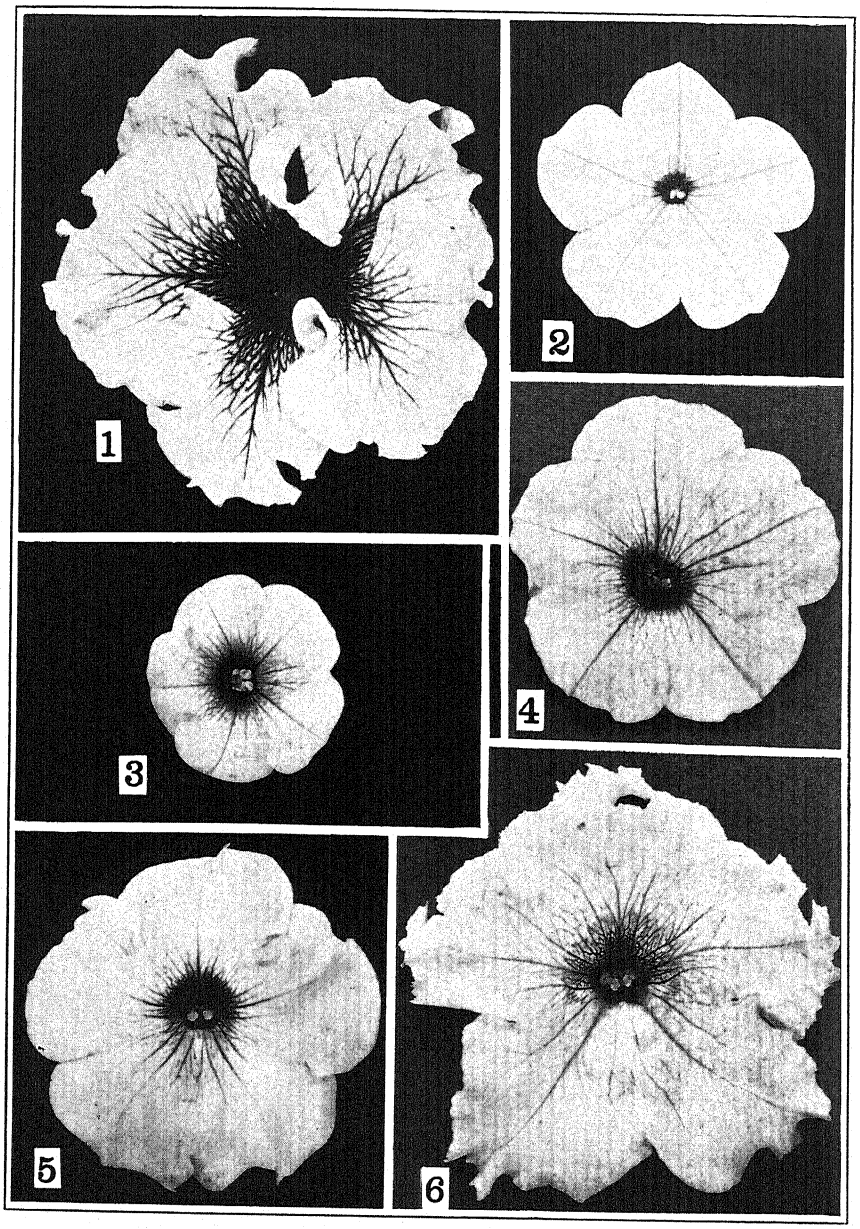

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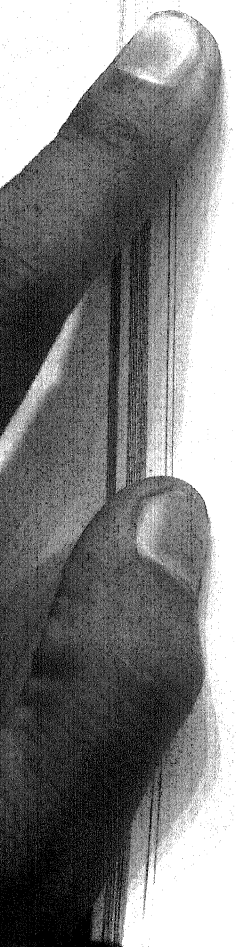

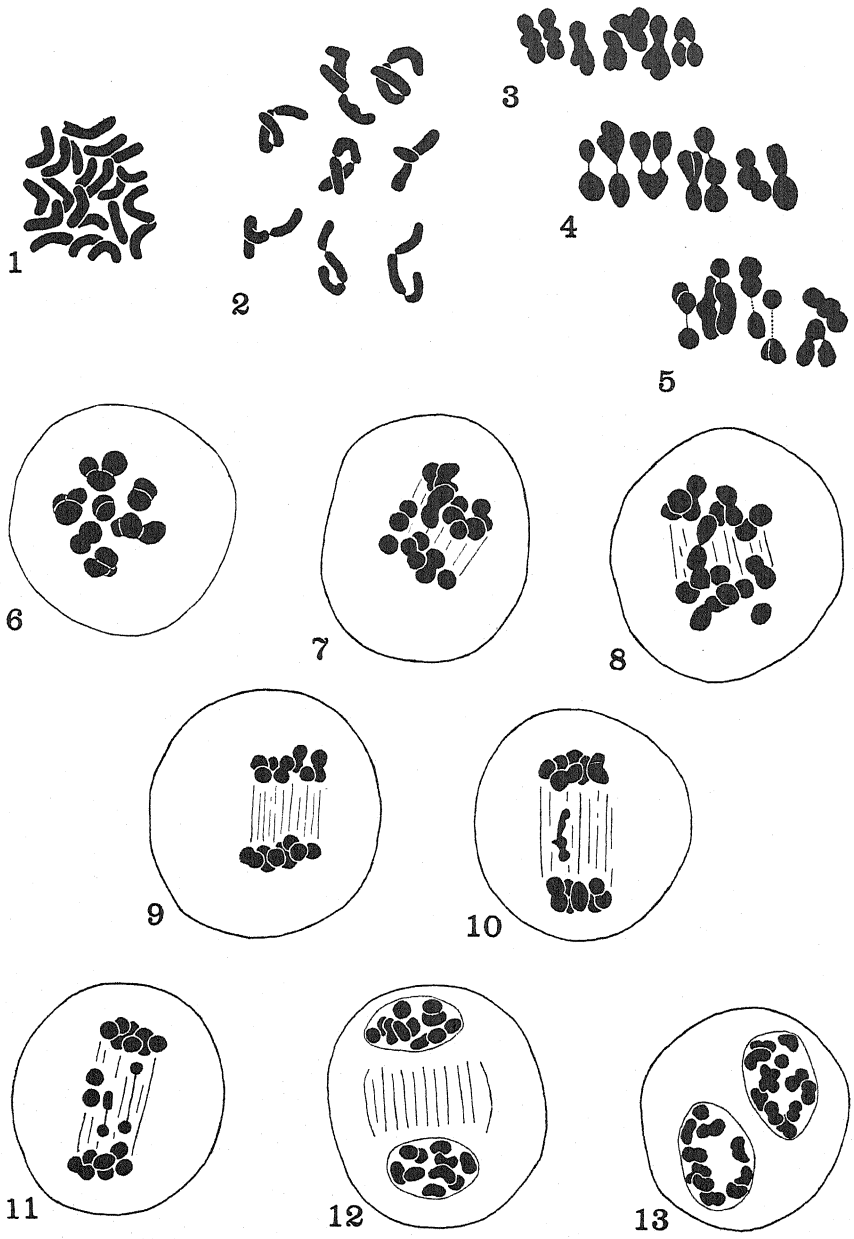

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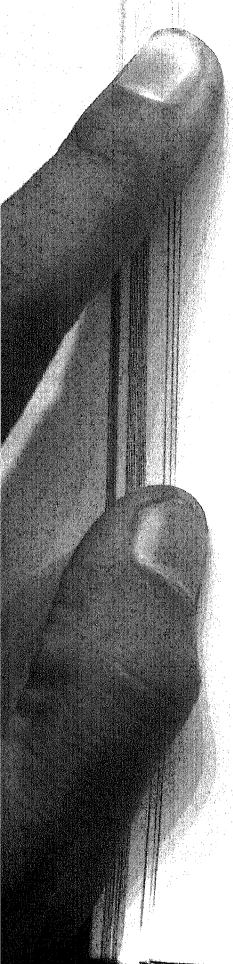

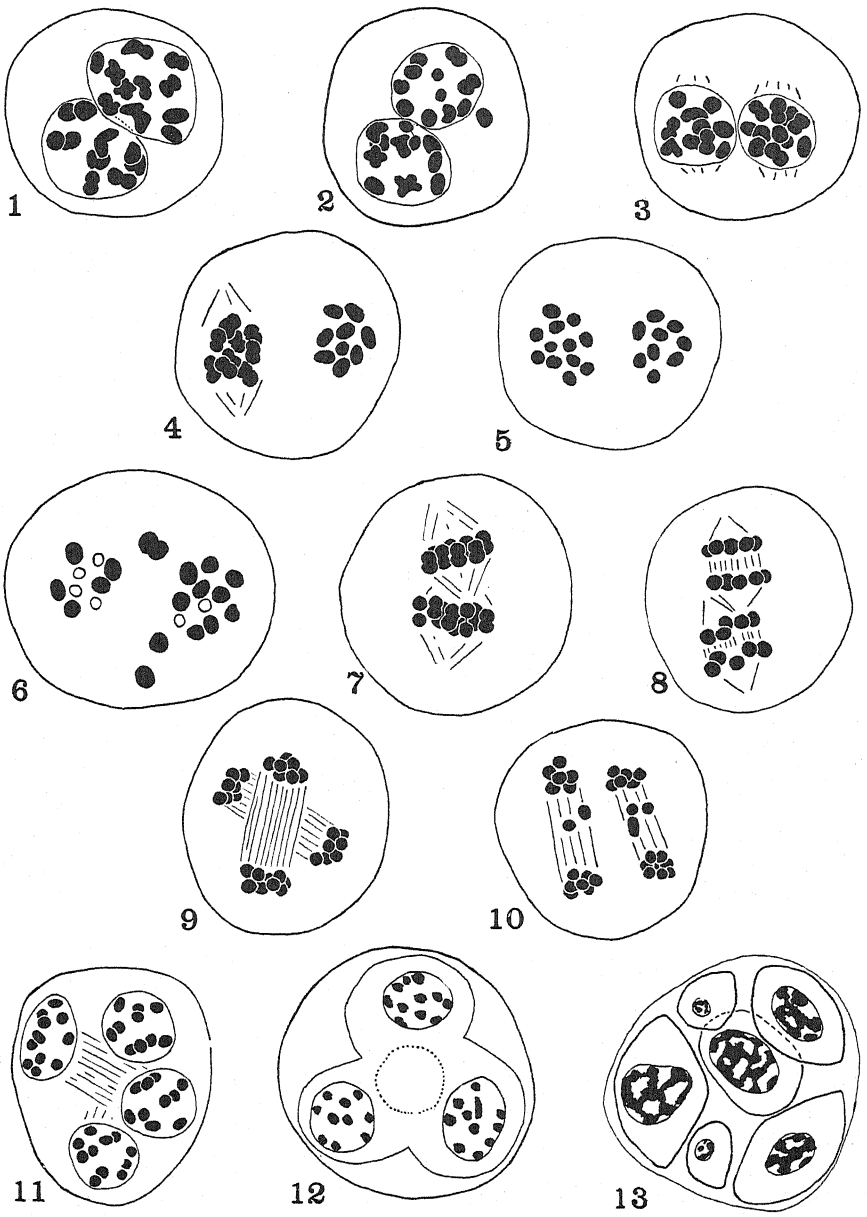

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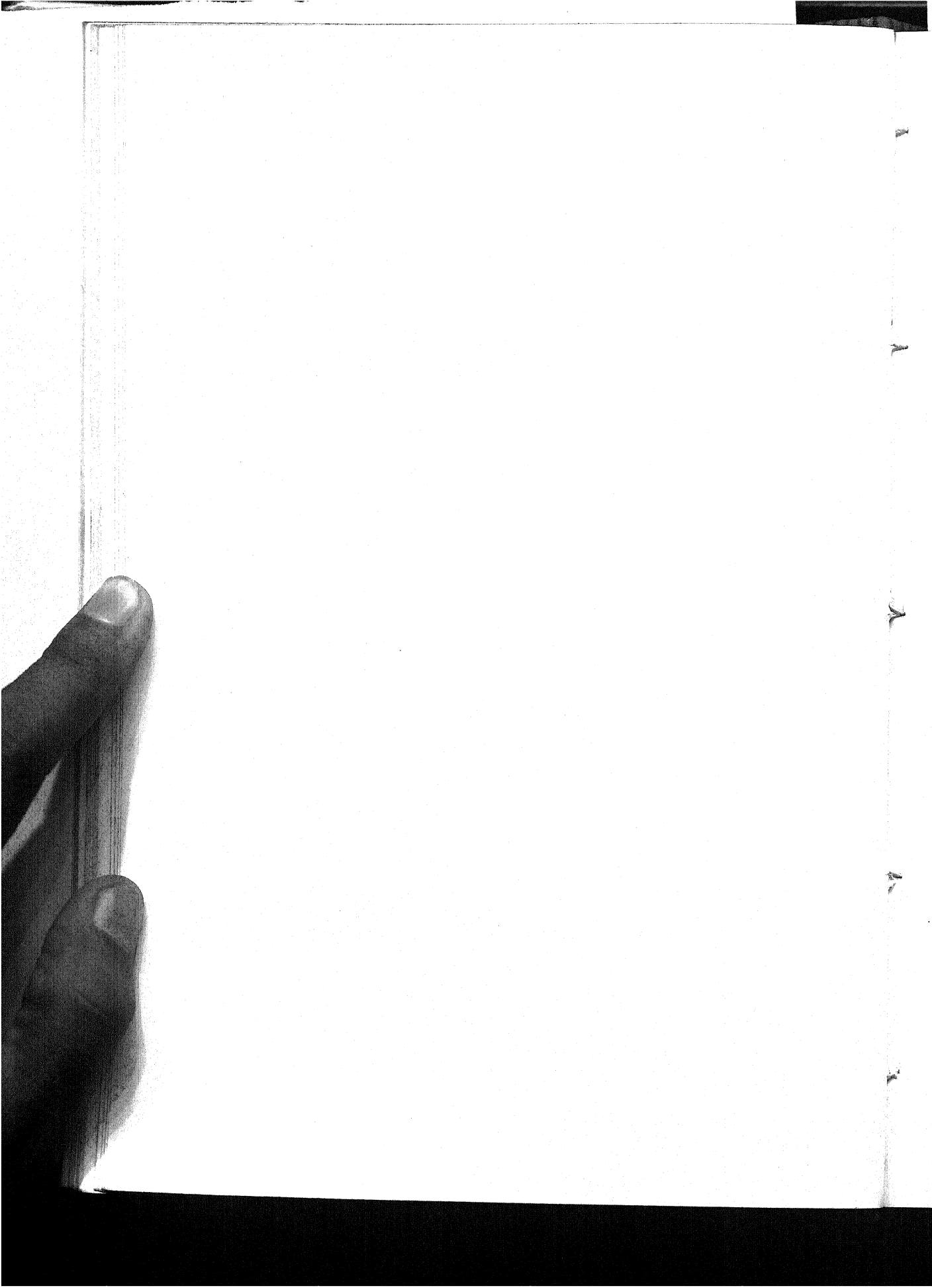

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DESCRIPTION OF PLATES

PLATE XXIII

FIG. 1. Pink flower from tetraploid parent of culture 266.*

FIG. 2. Flower from *Petunia axillaris*, the pollen parent of all the triploid plants.

FIG. 3. White flower from triploid plant 264–1. It is smaller than the flowers of either parent.

FIG. 4. Reddish-purple flower from triploid plant 264–10.*

FIG. 5. White flower from triploid plant 264–5.

FIG. 6. Lavender flower from triploid plant 266–4, showing resemblance to tetraploid parent.

* Due to the use of panchromatic film, the pink and reddish flowers appear quite light colored in the photographs.

PLATE XXIV

Cytology of the triploids

FIG. 1. Somatic chromosomes from nucellar tissue of ovule ($2n = 21$).

FIG. 2. Various types of chromosome association at diakinesis, taken from several different cells.

FIGS. 3–5. Side views of the chromosome complements of individual cells, at metaphase and early anaphase of the heterotypic division, to show the complete association of all the chromosomes into trivalents.

FIG. 6. Polar view of the heterotypic metaphase, to show the seven trivalents.

FIGS. 7–8. Anaphase of the heterotypic division, showing disjunction of the trivalent chromosomes.

FIG. 9. Late heterotypic anaphase to show the normal condition of regular chromosome distribution.

FIG. 10. Late anaphase, showing lagging bivalent chromosome in which the component parts are disjoining and each one is splitting.

FIG. 11. Late anaphase, showing two lagging univalent chromosomes which are splitting, and one lagging bivalent, the parts of which are disjoining.

FIG. 12. Telophase of the heterotypic division.

FIG. 13. Early interkinesis, to show the shape of the nuclei and chromosomes.

PLATE XXV

FIG. 1. Interkinetic nuclei.

FIG. 2. Late interkinesis, showing chromosomes which have split prematurely.

FIG. 3. Prophase of the homotypic division. The spindles are beginning to appear.

FIG. 4. Late homotypic prophase, showing a side view of one spindle and a polar view of the other.

FIG. 5. Homotypic metaphase, polar view, showing unequal numbers of chromosomes on each plate.

FIG. 6. Homotypic metaphase, polar view, to show chromosomes which have split prematurely and chromosomes which have become separated from the nuclei in earlier stages.

FIG. 7. Side view of the homotypic spindles at metaphase.

FIGS. 8–9. Successive stages in the homotypic anaphase, to show the regularity of chromosome distribution.

FIG. 10. Late homotypic anaphase, showing chromosomes lagging on the spindles.

FIG. 11. Telophase of the homotypic division.

FIG. 12. Cleavage of the pollen mother cell into pollen grains by progressive furrowing.

FIG. 13. A hexad, the supernumerary pollen grains being due to chromosomes or groups of chromosomes cut off in the cytoplasm following either of the meiotic divisions.

DEMONSTRATING THE LIFTING POWER OF TRANSPIRATION[1]

HIRAM F. THUT

(Received for publication October 11, 1931)

Transpiration and the cohesion of water are two important factors in the rapid rise of water in the conducting systems of stems. The problem of demonstrating these two factors has led to numerous experiments. In the present investigation several modifications of procedure have been devised and have been proved satisfactory. Living leafy twigs have been used to pull mercury columns to heights exceeding barometric pressure.

Previous investigators have used two general methods to demonstrate the plausibility of the cohesion theory of the rise of water in plants. In the first method a purely artificial system was used, the apparatus consisting of a porous evaporating surface attached to the top of a long glass tube. First the porous material and tube were thoroughly wetted and then the whole apparatus completely filled with water. The lower end of the glass tube was then dipped into a vessel containing mercury. On allowing the water to evaporate from the porous surface the volume of water decreases, and the mercury is pulled up the tube by the retreating water column. If the mercury column is lifted to a height exceeding barometric pressure then a definite pull is shown to exist in the water column. This experiment has been used to explain the rise of water in plants. The porous material is thought of as the leaf and the glass tube an element in the water-conducting system of the plant. The water is thus pulled up the trunk of the plant by the cohesive properties of the water column. Askenasy (1896), Ursprung (1913), Lubin (1926), Thut (1928), Otis (1930), and others have attempted to perfect such a set-up. The writer has described, in his paper just cited, an experiment in which a column of mercury was lifted 226.6 centimeters. This is equivalent to lifting a column of water approximately 100 feet.

In the second method actual living material was used as part of the apparatus. Leafy twigs were employed as evaporating surfaces. Only one successful set-up of this type, that of Boehm's (1893), has been reported in literature. His work, though often cited, has been neglected and probably doubted. Boehm successfully attached living *Thuja* twigs to capillary manometer tubes and by transpiration and cohesion of water lifted mercury columns to heights of 86.4 and 90.6 centimeters. By this method students of botany need not use an analogy, but can see by actual demonstration that a transpiring leafy twig does exert a pull on a water column.

[1] Paper from the Department of Botany, The Ohio State University, No. 282.

The writer has recently set up a series of experiments to substantiate Boehm's results and to make the experiment more practicable for class room demonstration. The several set-ups used were quite similar and may be summarized in two general descriptions. With a little experience and patience it is possible to set up a successful experiment with ordinary laboratory equipment. The materials necessary are: a living leafy twig 20 or more centimeters in length, a straight capillary tube 100 or more centimeters long, thick-walled, soft rubber tubing, a rubber band, 20 percent potassium hydroxide, chromic acid cleaning solution, a test tube, a slender pipette, clean mercury, and several beakers of hot water.

In setting up a demonstration the apparatus is prepared and assembled in the following fashion: The twig is cut from a tree or shrub and immediately placed under water and then a second cut is made several inches farther up the stem. The bark, including everything to the cambium, is peeled off for a distance of two or three centimeters from the cut end of the twig. This operation is performed under water. The base of the twig is then placed in a beaker of hot distilled water and from 4 to 8 centimeters of the base is boiled for nearly an hour. Care must be taken to protect the upper leafy portion of the twig so that it will not be injured by the heat.

An 8-centimeter portion of rubber tubing is boiled for an hour in 20 percent potassium hydroxide to remove possible fat-like materials. The tubing is then thoroughly washed with boiling distilled water. The long capillary tube is cleaned by running warm cleaning solution through the tube and following it with hot distilled water. The test tube is also thoroughly cleaned and then filled with hot distilled water.

The hot water, containing the clean rubber tubing, is cooled to about 40° C. by adding distilled water. This temperature makes it easier to handle the tubing. The twig is then transferred from the beaker in which the cut end has been boiled to the beaker containing the water with the rubber tubing. This transfer should be made as quickly as possible in order to avoid the entrance of air into the vessels. The cut end of the twig is then pushed into one end of the rubber tubing as far as the bark has been removed. This operation must be performed with both the rubber tubing and cut stem end submerged. The rubber tubing should fit tightly around the stem. This is possible if the stem and rubber tubing have been properly selected. The stem and attached rubber tubing are now removed from the water. Immediately the portion of rubber tubing covering the end of the stem is wrapped by using a rubber band. This is done to insure a perfect seal between the rubber and wood. As soon as the wrapping is completed the water that was retained in the rubber tubing is replaced by recently boiled water in order to eliminate possible air bubbles. This replacement is easily accomplished by holding the twig tip down and by inserting the water with a slender pipette.

The difficult part of the set-up of the demonstration is in attaching the twig plus rubber tubing to the long capillary tube so that the entire system is completely filled with water. The method usually used was to set one end of the capillary tube in a beaker of hot water and by suction at the other end the tube was filled with fairly hot water. This end may be closed with a finger tip until the tube is laid in a horizontal position and attached to the twig by forcing it quickly into the rubber tubing. Care must be taken to exclude all the air when this connection is made. Before the capillary tube was forced into contact with the stem end, the open end of the capillary was dipped into the test tube full of water. On bringing the glass tube and stem together the extra water in the rubber tubing flows into the capillary tube and removes all undissolved air from the lower end. The water in the test tube prevents undissolved air from entering the capillary tube.

One now has a twig attached by means of rubber tubing to a long capillary tube, with the tube completely filled with water and the lower end dipping into a test tube full of water. In this condition it is easily possible to move the set-up to the room where the demonstration is to be carried out. All that is needed to start a demonstration is rigidly to support the twig and tube in an upright position and pour clean mercury into the test tube. The transpiration of water from the leafy twig causes the water volume of the system to decrease. This decreasing volume causes the mercury to be pulled up the tube. As soon as the mercury column exceeds barometric pressure, the cohesion of water and the pull of transpiration are demonstrated.

A second way of making the connection between the twig plus rubber tubing and the long capillary tube was by using an elevated reservoir. The capillary tube was attached to an elevated reservoir containing hot water. The water was allowed to flow slowly through the tube and as soon as the capillary was entirely free of undissolved air, the twig plus rubber tubing were attached to the lower end of the capillary tube. The twig and capillary tube were then inverted and the tube dipped into a test tube full of water.

In the set-up just described the attempt was made to remove all undissolved air by boiling all materials. A second method of setting up the demonstration was also used with success. In this case the cleaned rubber tubing was attached to the unboiled wood of the twig immediately after the bark had been removed. The other end of the rubber tubing was attached to a suction pump and the twig permitted to hang tip downward. Sufficient water was included in the system so that the stem end of the twig was continually bathed in water. After the suction pump had removed most of the undissolved air from the cut stem end, the pump was detached and the rubber tubing filled with recently boiled, distilled water. The connection between the twig plus rubber tubing and the capillary tube was

made by the elevated reservoir method. After the capillary tube was washed free of undissolved air and the twig attached to the lower end of the capillary tube, the set-up was permitted to remain connected to the reservoir for several hours. This permitted the air-free water moving into the cut stem end of the twig to remove undissolved air. As soon as one wished to carry out a demonstration, the twig and capillary tube were inverted and the open end of the capillary dipped into a test tube containing water and mercury.

Not all set-ups proved successful. Twigs of some species of plants are more difficult to use than twigs of other species. This difference in the number of successes obtained by using twigs of different species of plants may be due to anatomical differences of the twigs or it may be that the right method of setting up for a demonstration has not been discovered. In practice several twigs and tubes were prepared for a demonstration. It requires only a little more time to set up several twigs and tubes than it does for one. With this number one may be certain of having at least one successful set-up.

After the mercury has been pulled up the tube, by transpiration, to a height at which the water column is ruptured, the twig may be used for a second attempt. The twig and rubber tubing should be detached from the capillary tube and the rubber tubing filled with water immediately after the break occurs. This prevents having much air pulled into the vessels or tracheids. On attaching the twig to the suction pump the air can be removed and the twig may be used for a second demonstration.

The time required for the transpiration from the leaves of a twig to pull the mercury to heights exceeding atmospheric pressure varied from fifteen minutes to six or eight hours. Many factors may influence the rate of rise so that the approximate time required can best be determined by running a preliminary test.

Table 1 contains some of the results that have been obtained. Included are the species of plant from which the twigs were obtained, the type of set-up used, the relative number of successes to total set-ups, the barometric pressure, the average height of the successes, the maximum height, and the maximum height in terms of water column for each species used. The height results have been corrected according to the following equation:

$$H = h + c + \frac{W_1 - W_2}{13.6}$$

H = Total height of mercury column.

h = Ht. of mercury meniscus in tube above the mercury level in the test tube.

c = Correction for mercury depression in glass tube.

W_1 = Length of water column from the mercury meniscus to the cut end of the stem.

W_2 = Height of water standing above mercury in test tube.

TABLE 1. *The Average and Maximum Height Results Obtained in the Successful Set-ups*

Species	Time of Year (1931)	Method of Set-up of Twig Ends	No. of Trials	No. of Successes	Barometer Reading During Experimentation Period, cm.	Av. Ht. Successful Trials, cm.	Maximum Ht. Obtained, cm.	Water Column Equivalent of Maximum Ht., feet
Thuja occidentalis	Apr. 11–13	Not boiled	12	4	75.0–75.6	83.3	90.3	39.9
Mahonia Aquifolium	Apr. 14–16	" "	4	2	74.5–75.0	88.0	91.0	40.2
Juniperus virginiana	Apr. 15–16	" "	10	3	74.5–75.0	89.6	101.8	45.0
Picea Abies	Apr. 30	" "	6	0	——	No success		
" "	Apr. 29–May 6 and Aug. 11	Boiled	24	13	73.7–74.9	84.9	94.7	41.8
Acer Negundo	May 7–10	Not boiled	16	0	——	No success		
" "	May 25–31	Boiled	10	5	73.6–74.8	83.7	97.1	42.8
Smilax hispida	May 29–30	Not boiled	3	1	74.4	——	86.0	38.0
Platanus occidentalis	May 30–June 2	Boiled	8	3	74.0–74.8	81.6	85.0	37.5

The results given in the table do not represent the highest results possible but represent those obtained while experimenting with the several methods. In the trials with twigs of *Thuja*, *Mahonia*, and *Juniperus* only the elevated reservoir plus suction pump method was used. In *Picea* and *Acer* both methods were used but only the boiled stem method proved successful. Failure to obtain successful results with the unboiled stems may have been due to the pith in the stems. Box elder, the *Acer* species studied, in particular has a large pith in the small twigs and was used deliberately to see if such twigs are usable. As the bark at the base of the twig had been removed and as the rubber tubing was only attached to the woody cylinder, failure on the part of the twig would be due to conditions either in the wood or the large pith. On placing the cut end of a twig of box elder in boiling water a large amount of air was forced out of the pith. After boiling the ends of several twigs for an hour, they were attached to tubes and these experiments proved successful. Twigs that had been boiled for an hour in the evening were allowed to stand over night in fresh, recently boiled, distilled water. These twigs on being attached to the capillary tubes the next morning gave even better results than those twigs that were used immediately. The twigs of box elder that had not been boiled apparently failed because it was impossible to remove a sufficient amount of the air from the pith. The unboiled *Picea* twigs also probably failed because of the air in the pith.

In *Smilax* the rubber tubing was attached directly to the cut stem end without the removal of tissue and recently boiled water was allowed to flow into the stem end for several hours before a demonstration was attempted. With two exceptions, the boiling method was the only one used on *Platanus* twigs. The two exceptions proved unsuccessful.

Other species of plants not mentioned were also used in these experiments. Since nearly all were used without boiling it is highly probable that failure to obtain mercury columns above atmospheric pressure were due to the undissolved air. By driving out the air in boiling water and giving enough time to permit the intercellular spaces in the pith to become filled with water, it seems highly probable that one could use leafy twigs of most woody plants in this experiment with success.

Summary

The lifting power of transpiration and the cohesion of water were demonstrated by a series of experiments. Living leafy twigs were used to pull mercury columns to heights exceeding barometric pressure. Several methods were devised and proved successful whereby twigs were attached to capillary tubes full of water. Loss of water from the twigs caused the water columns in the capillary tube to be pulled up the tube. These ascending water columns supported columns of mercury that exceeded atmospheric pressure. Twigs from the following plants were used success-

fully: *Thuja occidentalis*, *Mahonia Aquifolium*, *Juniperus virginiana*, *Picea Abies*, *Acer Negundo*, *Smilax hispida*, and *Platanus occidentalis*.

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SPOROGENESIS AND SEX DETERMINATION IN *BEGONIA SCHMIDTIANA* [1]

MARIA D. PASTRANA

(Received for publication June 23, 1931)

The following study of *Begonia Schmidtiana* Regel was originally undertaken as an investigation in sporogenesis, but because of the remarkable findings it later developed into an investigation of the problem of the determination of sex in a monoecious plant. The plant was selected from among a number of species of *Begonia* in cultivation at the Botanical Gardens of the University of Michigan chiefly because of the abundance of its flowers which made possible the collections of large amounts of cytological material from a single plant. The results lead to the exclusive study of this species, but it is hoped that investigations may be continued in other species of the genus and among related forms.

MATERIAL AND METHODS

The material was collected from the Botanical Garden chiefly during the summer months since at this season flowers are more abundant than at other seasons of the year.

Several fixing fluids were employed for different purposes. Strong Flemming's solution diluted with an equal part of water gave satisfactory preparations for some stages but it drew the chromosomes so closely together that accurate counts could not easily be made. A formol-acetic-alcohol mixture (commercial formalin, 2.6 cc.; glacial acetic acid, 1 cc.; 50 per cent alcohol, 40 cc.) gave preparations having good chromosome spacing, but with serious shrinkage of the cell contents. For chromosome counts and general purposes, the fluid that gave the best results was the following modification of Bouin's solution;

Bouin's solution	100.00 cc.
Chromic acid	.50 g.
Urea	1.00 g.

Material was left from 8 to 10 hours in the fluid, then rinsed with water and carried through a long series of alcohols. The stain most generally employed was iron-alum haematoxylin.

The following measurements will give an idea of the size of the nucleus and the nucleolus, and of the minute chromosomes of *Begonia:*

	Nucleus	Nucleolus
In the resting cells of the archesporium	3.95–5.52 μ	1.58–2.32 μ
In prophase of heterotypic mitosis	5.52–7.89 μ	1.58–2.32 μ
In synizesis	6.32–9.47 μ	2.37–3.16 μ

[1] Papers from the Department of Botany, University of Michigan, No. 359.

Measurements of the chromosomes shortly before metaphase of the heterotypic mitosis;

1st type =	2.37 μ long,	and	.592 μ wide
2nd "	1.975 " "	"	.685 " "
3rd "	1.58 " "	"	.79 " "
Odd chrom.	4.25 " "	"	.708 " "

Chromosomes are most easily measured shortly before they become arranged to form the equatorial plate on the heterotypic spindle. At metaphase of this mitosis the bivalents lie so closely together that it is difficult to distinguish them. At anaphase the chromosomes are more condensed than at metaphase.

Chromosome counts were made from the various parts of the plant substantially as recorded in the accompanying table.

TABLE 1. *Number of Chromosome Counts*

Chromosome Counts	♂ Flower 12 Chrom.	♀ Flower 13 Chrom.	Vegetative Sporophyte 13 Chrom.	Gametophyte		Endosperm 13 Chrom.
				♂ 6 Chrom.	♀ 6 or 7 Chrom.	
Growing point	50	50	50			
Developing petal	5	10				
Pedicel	10	10				
Developing stamen	50					
Epidermis of anther	10					
Tapetum	3					
Bract		10				
Developing ovule		100				
Nucellus		5				
Embryo			20			
Young leaves			30			
Root tip			20			
Hair			3			
Metaphase of heterotypic mitosis	300	200				
Anaphase of heterotypic mitosis	200	75				
Interkinesis	75	50				
Homoeotypic metaphase	150	100				
Microspore				100		
Megaspore					50	
Developing egg					15	
Endosperm						30

THE CHROMOSOMES OF THE STAMINATE AND PISTILLATE FLOWERS

Begonia Schmidtiana has chromosomes of 4 types when classified according to size and all are more or less curved like the letter "C." Four chromosomes (two pairs) are wider and longer than the others, four chromosomes (two pairs) are medium in size, and another four (two pairs) somewhat shorter than those of medium length and slightly broader. These three types are present in all parts of the plant. The fourth type is unpaired and

because of its absence in the staminate flower, it seems probable that this chromosome has a bearing on the determination of sex. It is coiled in form, tapering to a point, and resembles a question mark (Pl. XXXI, figs. 60 *s*, 61 *s*).

The sporophyte of the plant therefore carries 13 chromosomes in all tissues excepting those of the staminate flower where the number is 12. Counts of 13 chromosomes have been made in the root tip (Pl. XXVI, fig. 1), the embryo (fig. 2), the hair of the stem (fig. 3), the tip of the stem that will become the pistillate flower (fig. 4), the tissues of the ovule before the megaspore is formed (fig. 5), the petal and all the other parts of the pistillate flower, and the bud of the stem.

The staminate flower carries 12 chromosomes in all of its parts as shown by the counts from the flower bud initial (fig. 6), petal (fig. 7), the epidermis of the anther (fig. 8), archesporium initial (fig. 9), pedicel, bracts, and other regions.

The staminate and pistillate flowers arise close together from a common axis (text fig. 1). By horizontal cell divisions this axis divides dichotomously to give rise to the staminate and pistillate flower buds (text fig. 2).

TEXT FIG. 1. The origin from a common axis of the staminate flower (*S*) and the pistillate flower (*P*).

TEXT FIG. 2. Older stages in the development of the staminate flower (*S*) and the pistillate flower (*P*), showing dichotomous arrangement.

The dichotomous arrangement is, however, not very evident due to the fact that the staminate flower grows and matures faster than the pistillate and often drops from the plant three or four days before the latter opens. At the point of separation of the staminate and the pistillate flower buds the

unpaired chromosome apparently does not divide as do the others but goes as a whole into the bud that is to become the pistillate flower. Hence the staminate flower bud is deprived of that chromosome.

Němec (1904), followed by Häcker (1904), Woycicki (1906), and Schiller (1909), first reported the occurrence of heterotypic divisions in somatic cells as the result of treatment with chloral hydrate and other chemicals, but so far as the writer is aware this type of somatic reduction of chromosomes has not heretofore been reported as normal behavior, and the conclusions of this paper have been reached only after examining numerous specimens and finding no exceptions to the above statement of chromosome counts. However, the possibility that such a reduction takes place has been suggested.

Bond (1915) studied the sex characters of abnormal *Begonia* flowers, and found among other things that the "instability of equilibrium in the primary sex elements" is associated with the instability in somatic tissues. Thus an abnormal floral bract occurs on the pedicel of an abnormal flower and marks the place where the separation of the staminate and pistillate flowers would have occurred normally. Evidence was offered to prove that segregation of the sexes occurred in the meristematic part of the flower stalk and that "sex differentiation is a matter of qualitative cell division in somatic as well as in germinal cells." He stated further that sex dimorphism depends upon the kinds of units involved and their period of occurrence. Thus, in dioecious plants the differentiating cell division occurs in the germ cell, in monoecious plants at a later stage during flower formation, and in hermaphrodite plants at a still later stage during the development of sex organs. Finally he suggested that "if future cytological research should associate the male (or the female) sex in the Begonias with the presence of an accessory chromosome, then the position taken up by that chromosome in the qualitative cell divisions which divide the growing cell into end and lateral daughter cells will determine the primary sex of the flower just as the passage of such an accessory chromosome into one of the two alternative germ cells determine the sex of the individual."

Shaffner (1919) found in the dioecious plant *Cannabis sativa* L. that when it is grown out of season, with deficient light on a shallow soil, the female heredity which was at first active became suppressed giving away to the male inheritance which then became active. He concluded that "the change takes place in the vegetative body and is plainly caused by an internal change of the physiological state or condition of the meristematic tissues from which the flowers are produced." This statement clearly signifies a segregation of the sexes at some point in the meristematic tissue of a dioecious plant that becomes monoecious due to the action of environment although there is considerable time between the expression of the two sexes.

In view of these conclusions the present findings in *Begonia* are not without the support of other investigators.

Microsporogenesis

The archesporium arises from the third layer of cells below the epidermis (fig. 10). This line of cells divides to form a layer on the outside and the sporogenous tissue within. The layer, now third under the epidermis (Pl. XXVII, fig. 11), divides lengthwise to form a fourth layer and this becomes the tapetum surrounding the sporogenous tissue. In some cases a second division takes place, making the tapetum the fifth layer from the epidermis.

As the sporogenous tissue develops, the epidermal layer of the anther, and to a lesser extent the one just below it, becomes impregnated with a yellowish substance. The third layer loses its cell contents, and the tapetum stains deeply. In mature anthers only the tapetum and the second layer together with the epidermis remain, because the other cells between are absorbed apparently with the growth of the tapetum.

The sporocytes in a locule are usually in the same stage of meiosis, which however is generally a different stage from those of other locules in the same anther. There is therefore a wide range of stages in a single male flower which contains about 28 small stamens. Usually the outer stamens of the group mature earlier than the inner ones.

Prophase of Meiosis

A mature microsporocyte ready for meiosis contains a large nucleus occupying about one half of the cell cavity (fig. 12). The nucleolus lies at one side of the nucleus, and in fixed material it is surrounded by a clear area (fig. 13). The nucleus likewise lies at one side of its cell. The asymmetrical position of the nucleus persists through all stages, even up to the pollen tetrads. The nucleus contains a fine chromatic reticulum (fig. 14).

Certain strands of this chromatic network thicken coincident with the absorption of others and deeply staining threads become established (fig. 15). At this time and in later stages it is clear that the thread system is attached to the nucleolus (figs. 14, 16). No pairing of threads has been observed at this period of meiosis.

Synizesis

The thickening and condensation of the threads continues and the thread system gradually draws away from the nuclear membrane (fig. 16). The nucleolus frequently elongates at the point where it is attached to the thread system, thus becoming somewhat pear-shaped. Material appears to pass from the nucleolus into the threads (fig. 17), and the nucleolus stains much less deeply indicating that it has lost much of its contents. Masses of chromatin appear around the nucleus in grape-like clusters (fig. 18). The rest of the thread system which has not thus condensed gathers towards the contracted mass (Pl. XXVIII, fig. 19), and the nucleus passes into the condition of mid-synizesis (fig. 20) with all of the chromatic material contracted around the nucleolus and generally at one side of the nucleus. This condition persists for a long time, perhaps longer than any other stages of meiosis.

Frequently, before synizesis, the nucleolus may appear like a top around which a thread has been wound; or, it may resemble a fan-shaped arrangement of the threads. These conditions support the view of a definite connection between the thread and the nucleolus as reported by Van Camp (1924) for *Clivia miniata*, and Cleland (1924) for *Oenothera franciscana sulfurea*.

The nucleus emerges from synizesis through the gradual expansion of the thread system, as is illustrated by the figures 21 and 22. Finally an apparently continuous thread becomes evenly distributed through the nucleus, and the nucleolus, now staining more deeply, passes to the center (fig. 23). This is the stage of the open spireme.

Synapsis and Segmentation of the Spireme

The spireme gradually thickens in certain regions between which the thread becomes thin as though material had passed into these denser regions (fig. 24). Some of the threads come to lie parallel, in certain cases apparently because the sides of the loops approach one another (fig. 25), and frequently these threads lie so closely together as to twist about one another (figs. 26, 27). This seems to be the period of synapsis, since at this time homologous chromosomes become associated in the preparation for the later bivalents.

The drawing out of the thinner portions of the thread results in a segmentation of the spireme with the thicker regions as centers of chromosome organization. It is difficult to count the segments because of their varied forms and because the process of condensation generally brings them into confused groups, but the number seems to correspond closely to the chromosome count of 6 bivalents. This is the stage of second contraction.

Diakinesis

This stage really begins during the period of second contraction when the paired threads may be recognized in a state of synapsis. The paired threads may still be observed while the chromosomes are in an early stage of condensation (figs. 26, 27). Later it becomes evident that each thread gives rise to a chromosome (fig. 28). This is the true stage of diakinesis.

Although the chromosomes gradually become shorter and thicker they still retain a spiral form. Remnants of the linin threads may be observed attached to chromosomes (fig. 29). The chromosomes now become evenly distributed throughout the nuclear cavity and as they continue to shorten the spiral twists disappear. Finally the chromosomes assume their normal curved form and the association in pairs becomes less evident (Pl. XXIX, fig. 30).

Spindle Formation

After the condensation of the chromosomes the nuclear membrane gradually breaks down. The nuclear space becomes oval in form and spindle fibers begin to appear (fig. 31), becoming conspicuous even before the meta-

phase stage. For this reason when the chromosomes are grouped on the spindle in preparation for metaphase (fig. 32), they may give the impression of irregular arrangement. This irregularity is, however, shortly followed by a grouping of the chromosomes in pairs (figs. 33, 34) preliminary to the metaphase figure.

Metaphase and Anaphase

After the spindle is formed the homologous chromosomes are found closely paired, and arranged on the equatorial plate. The association is so intimate as sometimes to conceal the identity of each homologue (figs. 35, 36), and the individual chromosomes of each bivalent are difficult to recognize. They are most clearly shown in polar views of the equatorial plate (fig. 37) in metaphase.

The arrangement of the chromosomes follows the usual pattern for the heterotypic mitosis and anaphase presents no exceptional features. One half of the chromosome complement passes to each pole of the spindle (fig. 38) as reduction takes place. Metaphase and anaphase may frequently be found in the same locule of the anther.

Telophase and Interkinesis

During anaphase of the heterotypic mitosis there appears the first evidence of a lengthwise fission of each chromosome in preparation for the homoeotypic division (fig. 39). Therefore six split chromosomes are carried into telophase. The halves of the chromosomes at first remain attached at their ends forming V's but later become quite separated from one another (fig. 40) except for thread connections that form an imperfect network within the nucleus (figs. 41, 42).

Interkinesis is a well-defined period between the heterotypic and the homoeotypic mitoses during which the nucleus again acquires a nucleolus. The halves of the split chromosomes lie so far apart that 12 chromosomes are easily counted. It is difficult to recognize that association of the halves of split chromosomes which is so commonly found at this period of meiosis in many plants.

Homoeotypic Mitosis

The homoeotypic mitosis presents no important distinctive features. The halves of split chromosomes become associated during prophase (Pl. XXX, fig. 43) and at metaphase come so closely together that they are not easily recognized (fig. 44), and then are distributed, 6 chromosomes passing to each pole (fig. 45). During telophase the chromosomes elongate (fig. 46) and presently form a close network of delicate threads.

Cytokinesis

During synizesis the cytoplasm starts to withdraw from the cell wall. Contraction begins at the corners of the cell (Pl. XXVIII, fig. 19), thus rounding off the edges of the protoplast, which then becomes much smaller in

volume in comparison with the size of the nucleus. By a later breaking down of the primary walls the cells of the sporogenous tissue become separated and finally lie freely in the fluid of the pollen sac. A secondary wall gradually forms and thickens as sporogenesis proceeds until it becomes a conspicuous structure in later stages of meiosis (fig. 47). Castetter (1925) found such a wall in *Melilotus* which he thought to be a callose secretion of the sporocyte.

Cytokinesis results from a cleavage of the cytoplasm by furrowing, similar to that described by Farr (1916) in *Nicotiana*. In the telophase of the heterotypic mitosis, thickenings clearly visible under the low power objective were observed at the median plane of the spindle (fig. 47). These however are not concerned in the formation of a wall. Following the homoeotypic mitosis fibers were found connecting the four daughter nuclei (fig. 46). Vacuolization occurs in the equatorial plane of these fibers (fig. 48) as has been observed by Castetter (1925) in *Melilotus*. Furrows from the periphery cut inward through these vacuoles and, meeting at the center of the cell, divide the protoplast into four tetrahedral daughter cells (fig. 49). Extensions of the secondary wall follow the planes of the cleavage furrows. The secondary wall is of temporary nature since it disorganizes before the pollen grains reach maturity (fig. 50).

Megasporogenesis

Development of the Ovule and of the Integuments

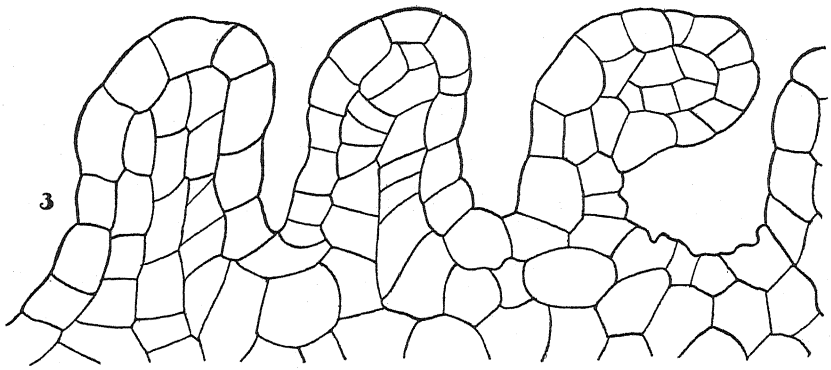
During the formation of the ovules the placenta stains deeply because the cells become filled with globules of food material. Swellings which later become ovules arise subepidermally from the placenta. A young ovule is at first an outgrowth composed of a few rows of cells (text fig. 3). Rapid cell divisions at its tip differentiate an enlarged upper portion from a thin stalk. Further divisions considerably enlarge the tip which then begins to curve towards the placenta (text fig. 4). As the ovule bends, the epidermal cells in its dorsal region increase in size and the inner and outer integuments arise from this dorsal portion of the ovule (text fig. 5).

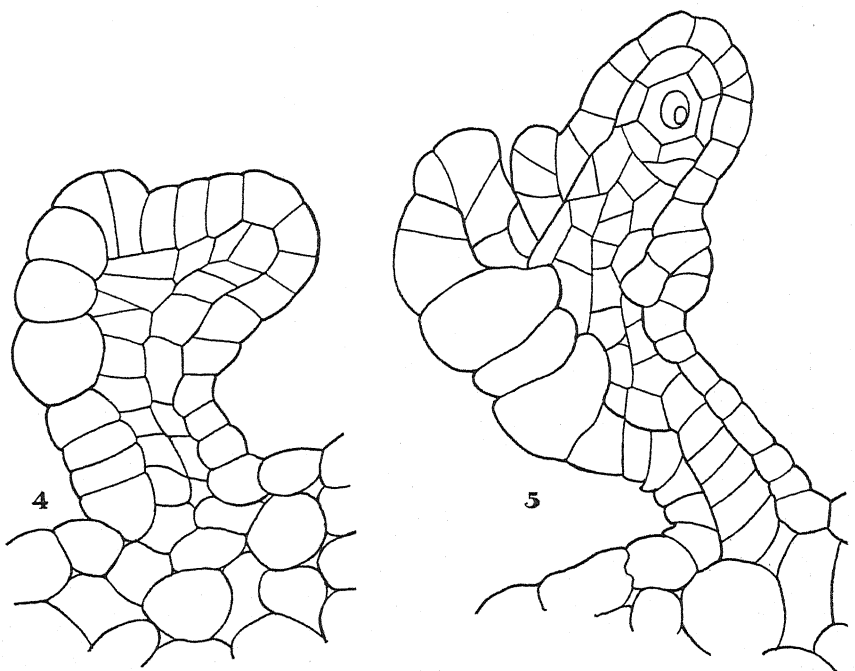
The outer integument grows more rapidly and its outer layer is composed of very large cells. The inner integument made up of small cells develops more slowly but finally grows beyond the tip of the nucellus leaving only a minute micropyle. The final result of the development is an anatropous ovule (text fig. 6).

Meiosis

The megasporocyte is differentiated in the nucellus as the second cell below the epidermis and is easily recognized after the integuments begin to appear (text fig. 5). It becomes a large cell as the ovule develops. The history of meiosis is the same as in the microsporocyte except that in the megasporocyte the odd chromosome is present. This chromosome can be

recognized at the metaphase of the heterotypic mitosis as an unpaired coiled chromosome (Pl. XXXI, figs. 60 *s*, 61 *s*).

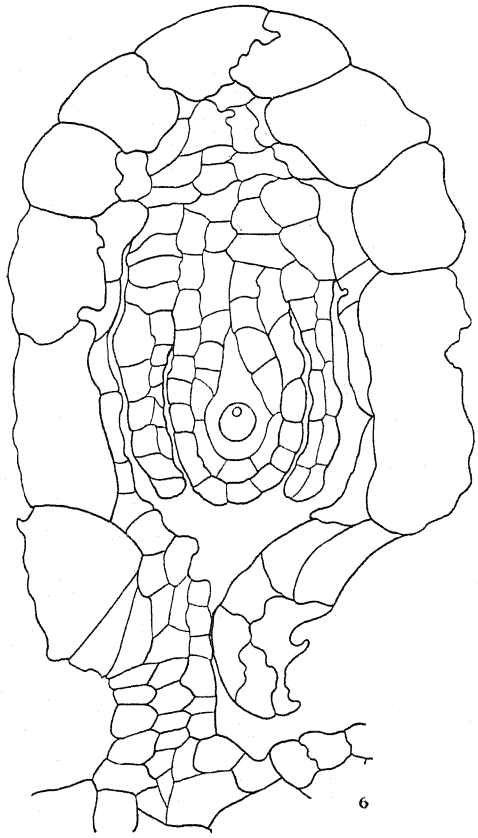




TEXT FIG. 3. Early stages in the development of the ovule.
TEXT FIG. 4. Older ovule beginning to curve, integument starting to develop.
TEXT FIG. 5. Ovule with definitely formed megasporocyte and integument initials.

The early stages of meiosis take place during the development of the ovule and the heterotypic metaphase does not occur until the ovule is at least as old as that shown in text figure 6. The prophase of meiosis follows the same history as in the microsporocyte. There is a long period when the nucleus is in synizesis (Pl. XXXI, figs. 51, 52) followed by the formation of a

spireme on which thickenings may be observed where the chromatin material seems to collect (fig. 53). Loops are developed and the threads pair with one another (fig. 54). Segmentation of the spireme follows as a result of the drawing out of the thinner portions of the threads. There is then a second contraction of chromatic material (fig. 55) during which synapsis is continued by a further twisting of the paired threads (fig. 56). Soon the pairs



TEXT FIG. 6. Ovule with megasporocyte in early stage of meiosis, outer integument covering the inner integument.

loosen, each thread becoming a chromosome which at first retains the spiral twists of synapsis (fig. 57).

The chromosomes then shorten and assume their usual form (fig. 58). During the formation of the spindle the homologous chromosomes become arranged in pairs (fig. 59). Metaphase follows and in this stage the bivalent chromosomes are easily counted and their form and size determined, the odd chromosome *s* being easily recognized by its spiral form (figs. 60, 61). A lengthwise splitting of the chromosomes in preparation for the homoeotypic mitosis gives V-shaped structures during telophase that persist into

interkinesis (fig. 62). Soon the homoeotypic division takes place (Pl. XXXII, figs. 63, 64) and the four megaspores are formed.

Our chief interest in this history concerns the behavior of the odd chromosome which makes the count in the megasporocyte 13 in contrast with the count of 12 in the microsporocyte.

During anaphase of the heterotypic mitosis when the homologous chromosomes have segregated, the odd chromosome is found to pass to the pole nearest the micropyle. Because of the splitting of the autosomes the two nuclei following the heterotypic mitosis contain 13 and 12 chromosomes, respectively (fig. 62). These two nuclei usually divide simultaneously in the homoeotypic mitosis (fig. 63), although the mitosis nearest the micropyle may proceed more rapidly (fig. 64). The four megaspores which result are arranged in a line (fig. 65), the two inner nuclei of the series having six and the two nearest the micropyle having seven chromosomes. The egg nucleus of the embryo sac carries the odd chromosome.

Discussion

As in other characters of an organism, sex determination depends on two principal factors, heredity and environment. There seems little doubt that both factors take part in the determination of sex, in spite of some extreme views which recognize only one of the factors. Many investigations have been made on the role played by these factors, with remarkable results. A few examples will be cited and the influence of environment on sex will be considered first.

As early as 1879 Prantl observed that strong light caused the production of female prothallia in ferns, while weak light caused the production of male prothallia. Later in 1881 he showed that strong light and abundant food supply induced the production of archegonia and the reverse condition the production of antheridia in *Equisetum*. Buchtien (1887) supported these conclusions, but Darling (1909) pointed out that the prothallia with which Prantl worked were not strictly dioecious but had only a tendency to dioeciousness. The important studies of Klebs (1896) proved that environment determined the production of vegetative branches, zoöspores, or gametes in certain algae. Cutting (1910) showed that sex in *Marchantia*, apparently so stable, is not wholly determined by segregation. That sex in ferns is influenced by environment has been shown by the experiments of Wuist (1913) on *Onoclea*, Nagai (1915) on *Osmunda* and *Asplenium*, and Czaja (1921) on several other species.

Shaffner (1919–1928) has made extensive reports on the influence of environment on sex, studying the effects of age, nutrition, light, etc., in *Cannabis sativa*, *Morus alba*, *Salix amygdaloides*, *Humulus japonica*, *Thalictrum dasycarpum*, and *Arisaema Dracontium*. His conclusions were positively in favor of the environmental factors as modifiers of sex. He was able to reverse the sex of the dioecious plant hemp, and to produce intersexes

or only one sex by changing environmental conditions of the plant. However, he does not disregard the internal mechanism as a factor collaborating with environment in the determination of sex.

It is claimed by Ciesielski (1911) that the age of pollen has an influence on the sex of hemp, fresh pollen grains giving rise to seeds producing male plants, and old pollen grains to female plants. However, Bessey (1928) working with hemp found that the age of pollen had no effect on the sex of the resulting offspring.

That environment affects the chromosomes has been demonstrated by Belling (1924–1925) who showed that warmth decreased the number and size of chromosomes in *Cypripedium acaule*, and that cooling at night and changes of temperature for three years caused chromosomal mutations in *Uvularia*. Hirata (1927) claimed that sex in hemp is due to *XY* chromosomes and may be influenced by environment through its action on enzyms affecting sex.

In animals, the effects of environment on sex have also been studied. Riddle (1912) found that the kind and amount of food modified the sex of pigeons, and that eggs of small size, high water content, and small energy content are male-producing while those of opposite characters are female-producing. Hertwig (1912) working in frogs stated that over-ripe eggs produced an excess of male offspring and vice versa. Lillie (1917) reported for twins in cattle that the secretion of a certain hormone by a male embryo arrests the normal development of an accompanying female, producing the sterile "free martin." Minoura (1921) found that grafting of reproductive tissues changes the sex of the chick, but that grafts from liver and other non-sexual organs had no effect.

On the other hand, proofs that sex is determined through inheritance are overwhelming. The idea was first suggested by Mendel himself. The first demonstrations were made through animals.

In 1891 Henking observed additional chromatin material which he thought to be a nucleolus in half of the spermatids of *Pyrrhocoris*. Other investigators, McClung (1899), Paulmier (1899), and Montgomery (1901), reporting similar conditions in other insects such as *Anasa*, *Protenor*, and species of grasshoppers, discovered this body to be an accessory chromosome and McClung associated it with the determination of sex.

It was later found that sex determiners may occur in pairs, and may be located in the male or in the female individual. When the male carries the sex chromosome it produces two kinds of sperms, as in the *Lygaeus XX-XY* type described by Montgomery (1901). The female may behave in the same way, as in the moth *Abraxas grossulariata ZW-ZZ* type described by Doncaster (1913–1914), or the *ZO-ZZ* type in *Taloepora tubulosa* described by Seiler (1917). Cytological and genetic evidence has firmly established the presence of sex chromosomes in many animals and an extensive literature had been developed about the subject, recently reviewed by Schrader (1928).

Sex chromosomes have been located, and described as to their behavior, size, and shape, and environmental factors affecting them have been determined.

In plants the idea of a Mendelian inheritance of sex was early supported by Strasburger (1900), Bateson and Saunders (1902), and Castle (1903). In 1906, Blakeslee observed that sexual segregation occurred in *Phycomyces nitens*. This was shown by Burgeff (1914–1915) to be due to nuclear differences in the hyphae. The Marchals (1907) demonstrated that in certain mosses internal mechanism rather than environment determined sex and that segregation of the sexes occurred at meiosis. Correns (1907) found two kinds of pollen grains in the dioecious plant *Bryonia*, but only one kind of egg. Strasburger (1909) observed that of the four spores of a tetrad in *Sphaerocarpos* two developed male and two female gametophytes. It was suggested that the development of distinct male and female plants from the tetraspores of *Polysiphonia* indicates a segregation of sex factors in sporogenesis. These illustrations indicate that segregation of the sexes takes place in the life cycle of some plants through nuclear mechanism. Sex chromosomes in plants were, however, not identified until Allen (1917) discovered them in *Sphaerocarpos Donnellii*.

In certain dioecious angiosperms several investigators through genetical studies reported two types of pollen grains. Strasburger (1910) suggested the possibility of such condition. Correns (1907) in *Bryonia* and Shull (1910) in *Lychnis* demonstrated that male plants were heterozygous for sex.

In 1923 investigators working on four different dioecious plants demonstrated two types of pollen grains differing in the presence or absence of an accessory or sex chromosome, thus presenting the same situation as is found in certain animals. This was shown by Santos for *Elodea*, Kihara and Ono for *Rumex*, Winge for *Humulus*, and Blackburn for *Melandrium*. Later studies by Meurman (1925) have added several species of *Populus* to the list. These cytological studies on plant material give support to the suggestions of earlier workers, Strasburger (1900), Correns (1907), and Shull (1910), that sex is determined through a mechanism of inheritance.

In conclusion, the following quotations may serve to express the prevailing opinions on the determination of sex. Thomson (1913) states that "if nutritive and other environmental influences are operative, it is, in the main, by affecting the production and survival of sexually predestined germ-cells." Sharp (1926) expresses the opinion that "under ordinary conditions it is the genetic factors that are differential," and that "the effects of heredity and environment are not so much opposed as complementary." Wilson (1928) affirms that "investigations upon the nature of sex were long dominated by the preconceived notion that it is determined by conditions external to the germ, but the inadequacy of this view is now conclusively demonstrated. . . . It seems clear, however, that external conditions operate merely to incite the development of one set of sexual characters at the cost of the opposite set in an organism that is actually or potentially monoe-

cious or hermaphroditic." In dioecious organisms, "both the genetic and cytological evidence demonstrates the existence in the germ-cells of a definite internal mechanism that is adjusted for an automatic production of the sexes under identical external conditions. It is probable, therefore, that in so far as external conditions affect sex production it is in all cases through their influence upon the internal mechanism."

It is not the purpose of this paper to claim that sex inheritance is purely Mendelian in character, for although more generally heredity appears to determine sex, the results have been modified by experimental control of environmental conditions. The two factors go hand in hand and sex is determined by their interrelation. The internal factors depend on the structure of chromosomes subject to the influence of hormones and enzyms, and these in turn are influenced by the environment under which the plant thrives. The external stimuli may either be favorable or lethal and the latter may vary in toxic effects from a mild inhibition to actual destruction. There is an interdependence of the individual organs together with a relation to an outside world. Chromosomes are vehicles of sex determination, but are subject to outside influences. Confirmation of the latter conclusion does not disprove the former statement.

It is here proposed that the accessory chromosome present in the pistillate and absent from the staminate flowers of the monoecious plant *Begonia* is a sex determiner of a like nature to that found in the pollen or spores of several dioecious plants, although its critical behavior as a determiner of the sex in *Begonia* occurs with the differentiation of the flower initials rather than at meiosis. Since it has been shown that sex chromosomes or determiners are present in both animals and plants, it is but natural to expect that they should be present in such a highly evolved plant as *Begonia*.

When sex determiners are carried by the male gamete, a permanent separation of the sexes is assured as in the case of animals and dioecious plants. Sex is acquired by chance because two kinds of male gametes give two different zygotes in the fertilization of the sexually similar eggs.

However, since the sex chromosome present in the monoecious plant *Begonia* can not be found in the male gamete, for in that case a dioecious condition would result, it must therefore be present in the female gamete so that the offspring will be constant to the monoecious character of the species. Fertilization then can give only one type of plant because there is only one kind of pollen grain to fertilize one kind of egg, that which bears the sex determiner.

Segregation of the sexes must take place before the formation of the flower, for should it occur later during the formation of sex organs, the flower would be hermaphroditic as pointed out by Bond (1915). The monoecious condition in *Begonia* is due to the fact that the segregation of a sex chromosome occurs before the formation of the flowers and that the presence of the sex chromosome determines the development of a pistillate flower and

its absence the development of a staminate. Therefore the present findings in *Begonia* are what might be expected with respect to the segregation of the sexes and the final location of the sex chromosome in the egg.

The gap between sex segregation in dioecious plants, which occurs in the germ cells, and that in the monoecious plant, which takes place during floral development, seems a wide one. In the evolutionary stages this has probably been bridged by the occurrence of sex segregation at different points of location intermediate between the two. One might venture to speculate that this is one reason why sex in monoecious plants may be unstable; for example Bond (1915) showed the presence of abnormal flowers with characters of both sexes.

A detailed study of chromosome counts in the family Begoniaceae would be interesting. Heitz (1927) made such a study in 15 of the genera. He counted 29–32 chromosomes in "*Begoniastrum Schmidtiana.*" This must be a different species from the *Begonia Schmidtiana* of this paper. Heitz's counts indicate that his forms were polyploids, mostly tetraploids, and that the *Begonia Schmidtiana* under consideration here is a more primitive type. Moreover, it is interesting to note that his counts for species were not constant, thus: he records 36–42 chromosomes in *Pritzelia scandens*, 28–30 chromosomes in *Augustias Dregei* and *Magnusia metalica* and 24–28 chromosomes in *Petermannia isoptera*, *Huszia Baumanii*, *Magnusia conchaefolia*, *Donaldia ulmifolia*, and unclassified varieties of *Java assamica* and *mexicana*. These numbers indicate that they are tetraploids whose chromosome numbers are multiples of 6 and 7, the counts in *Begonia Schmidtiana* Regel. His other counts show that there is a great irregularity among the chromosome counts in different species. This is to be expected considering the extensive hybridization and cultivation that the plant has undergone.

Concerning sporogenesis and the life history in general, the following peculiarities deserve mention. Pairing of threads was observed after synizesis, in stages of the thicker spireme before diakinesis. Segmentation of the spireme is delayed until diakinesis. The connection of the nucleolus with the thread system is very evident.

The sex chromosome present in the sporophyte comes to enter the egg because it passes into the nucleus of the secondary megasporocyte that is nearest the micropyle. There seems to be a certain polarity of the heterotypic mitosis that directs this movement.

Summary

1. The sporophyte has 13 chromosomes of four types. One is unpaired, and is absent from the staminate flower, which consequently has 12 chromosomes in contrast to the pistillate flower with 13 chromosomes.

2. The unpaired chromosome is probably concerned with the determination of sex. It fails to enter the stem initial from which the staminate flower is developed.

3. The prophases of meiosis show a definite connection between the chromatic thread system and the nucleolus from which material passes into the threads.

4. Parallel threads were not observed until after synizesis. The parallel threads become nodulated before segmentation, the regions between growing thinner until separation is effected, and the nodules become centers of chromosome organization.

5. The number of segments seems to correspond to the number of bivalents found in the microsporocyte, that is, 6 pairs. Later, in diakinesis, the segments are recognized as 6 pairs of twisted threads.

6. Diakinesis begins during the period of second contraction when the paired threads may be observed in a state of synapsis.

7. The association of the homologous chromosomes at metaphase of the heterotypic mitosis is sometimes so intimate as to conceal their identity in the bivalents.

8. The first evidence of a lengthwise division of each chromosome in preparation for the homoeotypic division appears during anaphase of the heterotypic mitosis.

9. During interkinesis the halves of the split chromosomes lie so far apart that the 12 chromosomes are easily counted.

10. The nucleolus, which disappears before the heterotypic mitosis, reappears rather suddenly during interkinesis as though it might be a reservoir of chromatin supply.

11. Cytokinesis is accomplished by cleavage furrows that cut inward from the periphery through vacuoles that lie between the four nuclei following the homoeotypic mitosis. Extensions of a secondary wall follow the planes of the cleavage furrows, but this wall disorganizes before the pollen grains reach maturity.

12. The megasporocyte is differentiated at the tip of the small nucellus. The details of meiosis in megasporogenesis are essentially the same as in microsporogenesis.

13. The odd chromosome is easily recognized at metaphase of the heterotypic mitosis by its spiral form. It passes to the pole nearest the micropyle with 6 of the 12 autosomes.

14. Because of the splitting of the autosomes the two nuclei of the interphase of megasporogenesis following the heterotypic mitosis have 13 and 12 chromosomes, respectively.

15. Following the homoeotypic mitosis the four megaspores are arranged in a line, the two nuclei nearest the micropyle having 7 chromosomes each, and the two inner nuclei 6 chromosomes. The egg nucleus of the embryo sac carries the odd chromosome, making a total of 7 chromosomes in its set.

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EXPLANATION OF FIGURES

All drawings were made with the aid of a camera lucida under the Zeiss apochromatic oil immersion, pri. mag. 120, N.A. 1.3, with compensating ocular × 20, draw tube at 171 mm. Figures reduced one-third in reproduction.

PLATE XXVI

FIG. 1. Cell from root tip, 13 chromosomes.
FIG. 2. Cell from the embryo, 13 chromosomes.
FIG. 3. Cell from the hair of the stem, 13 chromosomes.
FIG. 4. Cell from the pistillate flower initial, 13 chromosomes.
FIG. 5. Cell from the nucellus of ovule, 13 chromosomes.
FIG. 6. Cell from the staminate flower initial, 12 chromosomes.
FIG. 7. Cell from the petal of staminate flower, 12 chromosomes.
FIG. 8. Cell from epidermis of the anther, 12 chromosomes.
FIG. 9. Cell from archesporial initial, 12 chromosomes.
FIG. 10. Archesporium in anther showing its origin from the third layer of cells below the epidermis.

PLATE XXVII

FIG. 11. Sporogenous tissue with surrounding tapetum.
FIG. 12. Resting cell of the sporogenous tissue.
FIG. 13. A later stage with a delicate chromatin network.
FIG. 14. Formation of chromatin thread attached to the nucleolus.
FIG. 15. A definite spireme.
FIG. 16. Thread system entering synizesis.
FIG. 17. Early synizesis showing material passing from the nucleolus into the threads.
FIG. 18. Further condensation of chromatin material.

PLATE XXVIII

FIG. 19. Mid-synizesis showing the contracted thread system.
FIG. 20. Mid-synizesis.
FIGS. 21 AND 22. Emerging from synizesis.
FIG. 23. Open spireme.
FIG. 24. Chromatin nodules on spireme.
FIG. 25. Pairing of threads.
FIG. 26. Paired threads twisted.
FIG. 27. Second contraction, twisted threads.

FIG. 28. Diakinesis, the chromosomes in pairs.
FIG. 29. Univalent chromosomes following diakinesis.

PLATE XXIX

FIG. 30. Further condensation of chromosomes.
FIG. 31. Beginning of spindle formation.
FIG. 32. Homologous chromosomes becoming arranged in pairs on spindle.
FIG. 33. Homologous chromosomes closely paired.
FIG. 34. Polar view of equatorial plate showing pairing of univalents.
FIGS. 35 AND 36. Metaphase of the heterotypic division, the univalents closely paired.
FIG. 37. Polar view of later metaphase, the univalents beginning to separate.
FIG. 38. Anaphase of heterotypic mitosis.
FIG. 39. Late anaphase showing splitting of the chromosomes.
FIG. 40. The daughter chromosomes separating after the split in late anaphase.
FIGS. 41 AND 42. Interkinesis showing univalent chromosomes and remnants of thread connections.

PLATE XXX

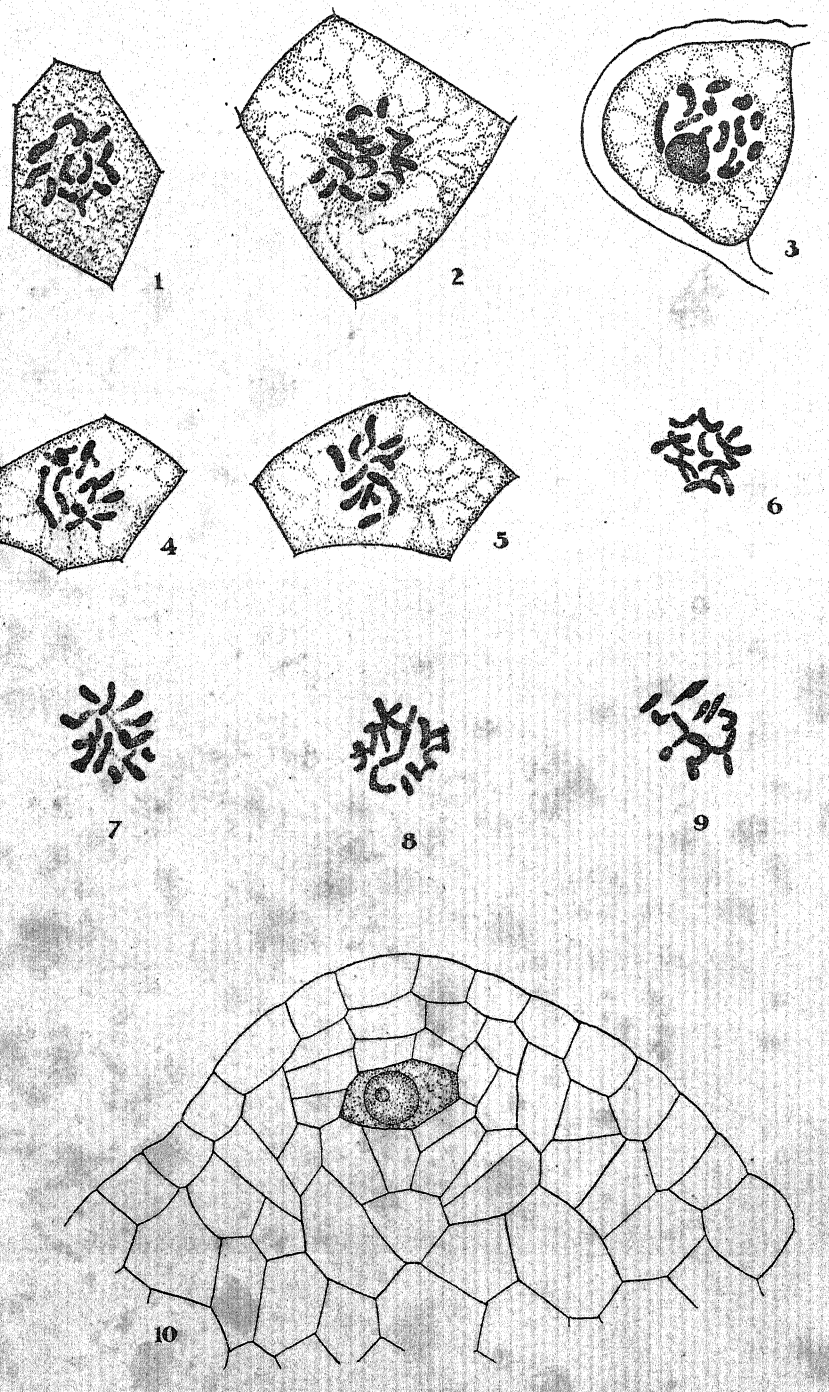
FIG. 43. Daughter chromosomes becoming associated preliminary to the homoeotypic division.
FIG. 44. Homoeotypic metaphases.
FIG. 45. Homoeotypic anaphases.
FIG. 46. Nuclei of the tetrad with connecting fibers.
FIG. 47. Secondary wall formed inside the primary wall.
FIG. 48. Tetrad showing vacuolization at the equatorial planes.
FIG. 49. Secondary wall following the furrows that have cut out the microspores.
FIG. 50. Tetrad of microspores.

PLATE XXXI

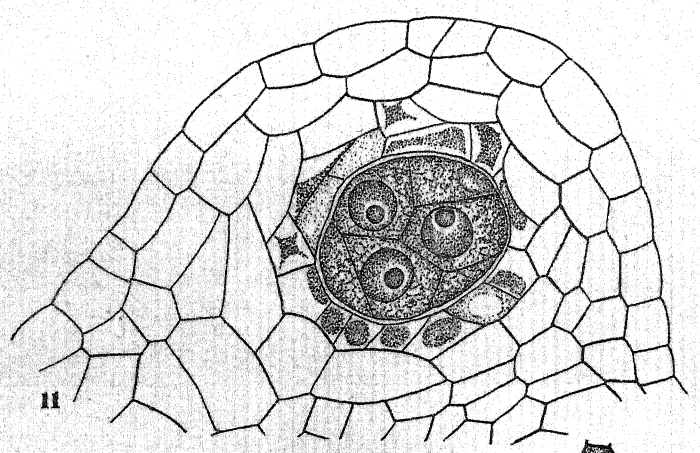
FIGS. 51 AND 52. Synizesis in the megasporocyte.
FIG. 53. Spireme with chromatin nodules, following synizesis.
FIG. 54. Pairing of threads.
FIG. 55. Second contraction of chromatin material.
FIG. 56. Diakinesis, the chromosomes in pairs.
FIG. 57. Univalent chromosomes following diakinesis.
FIG. 58. Further condensation of chromosomes.
FIG. 59. Homologous chromosomes becoming arranged in pairs on the spindle.
FIGS. 60 AND 61. Metaphase of heterotypic division in the megasporocyte. *s*, odd chromosome.
FIG. 62. Interkinesis in the megasporocyte.

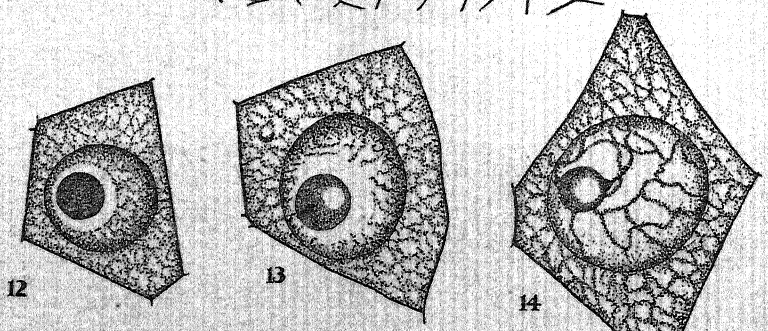
PLATE XXXII

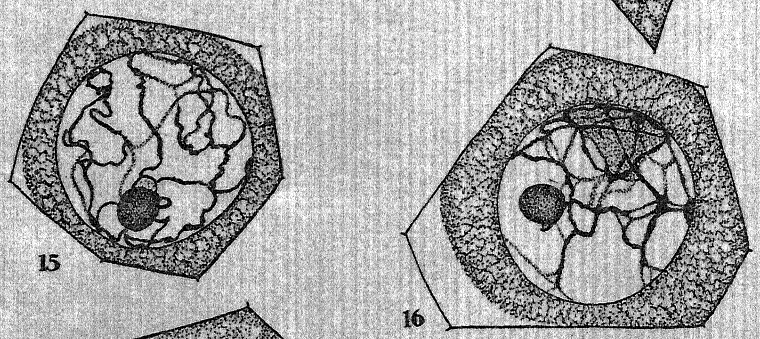
FIG. 63. Metaphases of the homoeotypic division showing 6 chromosomes on one spindle and 7 chromosomes on the other.
FIG. 64. Metaphase of homeotypic division in one cell, anaphase in the other.
FIG. 65. Megaspores (tetrad) showing relation to two large cells of the nucellus at the micropylar end.

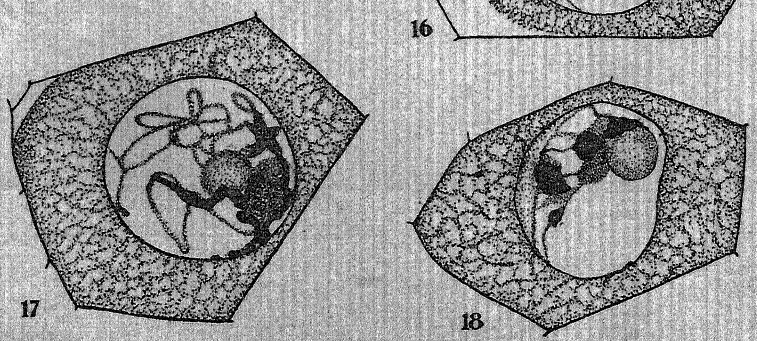


PASTRANA: SPOROGENESIS IN BEGONIA









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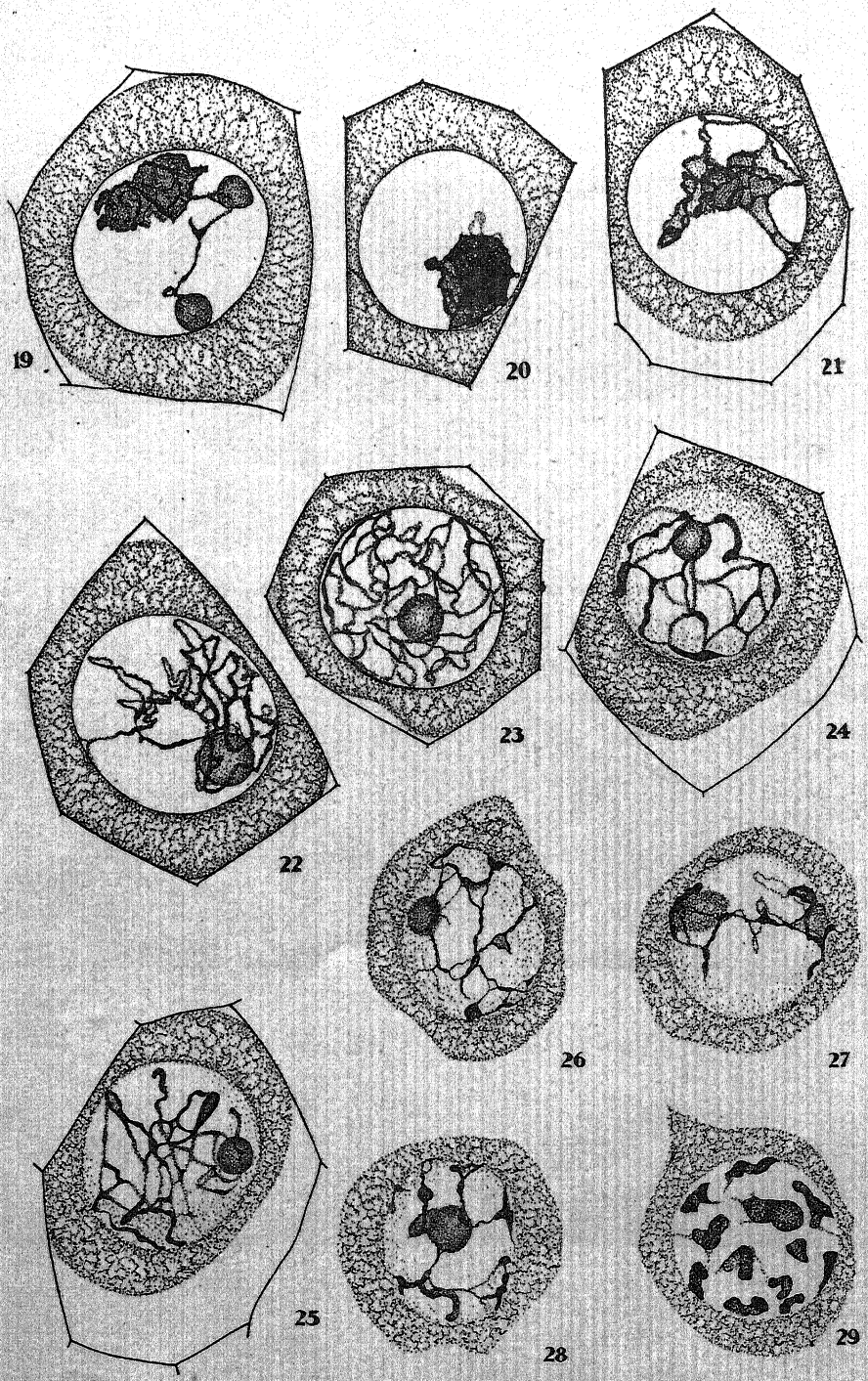

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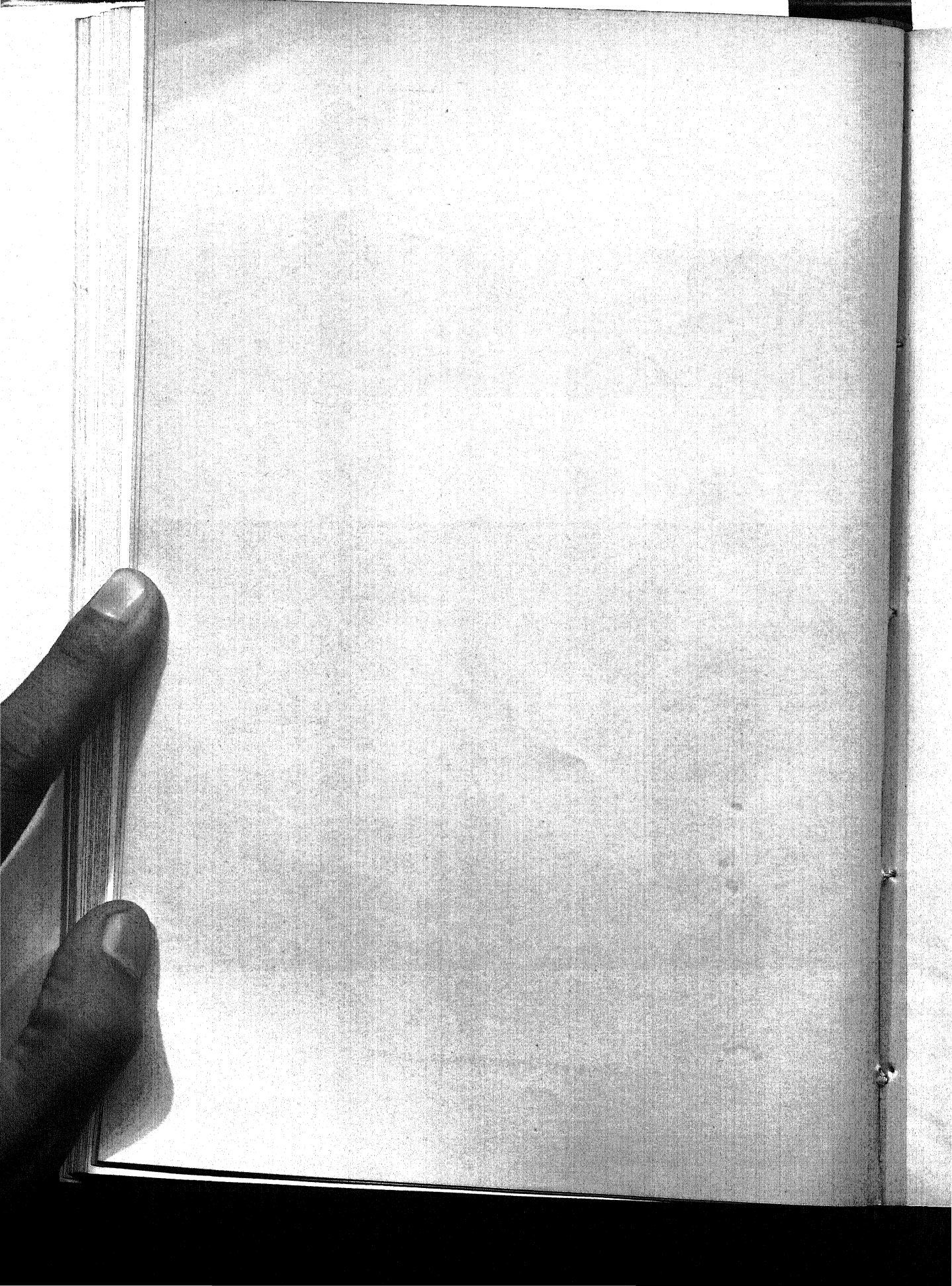

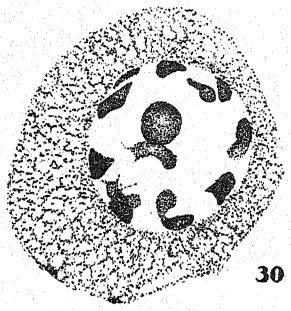
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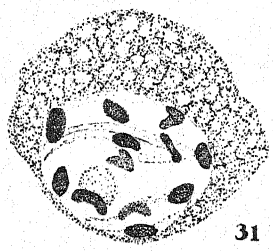
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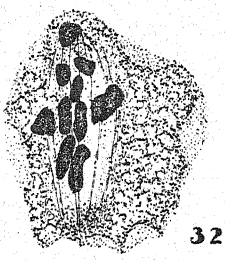
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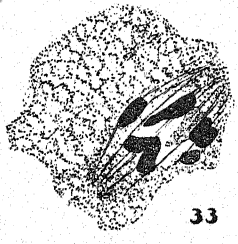
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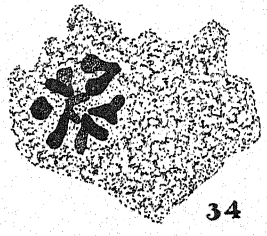
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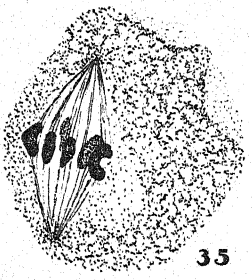
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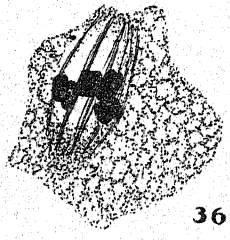
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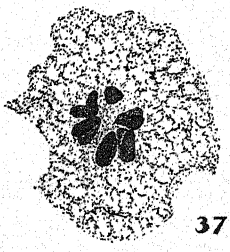
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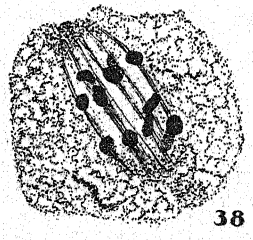
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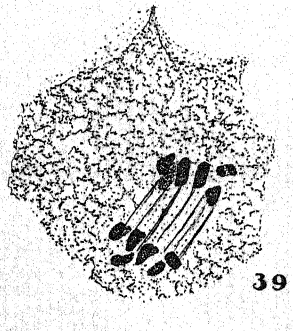
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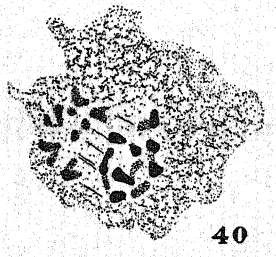
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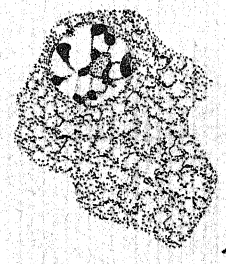
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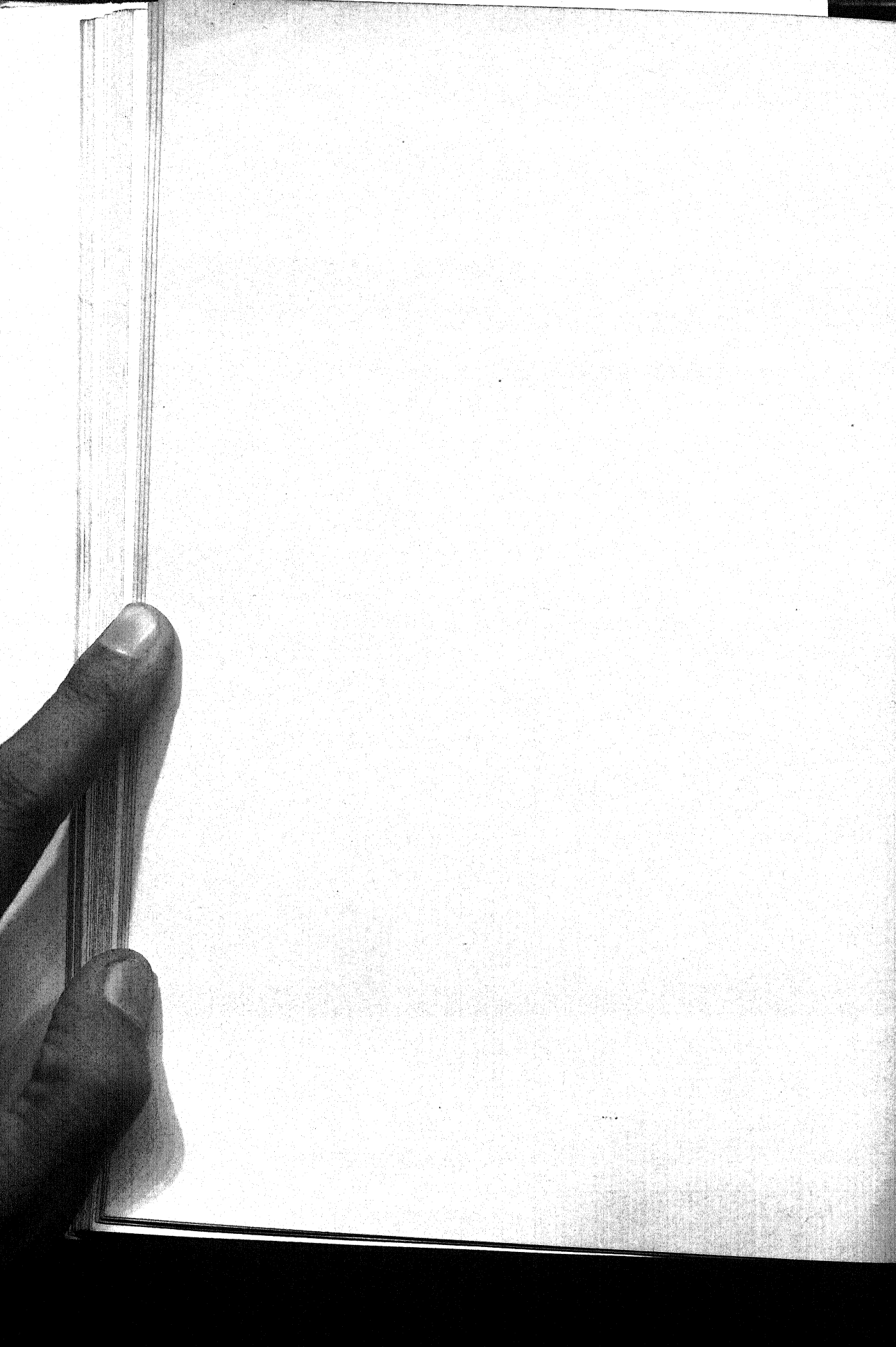
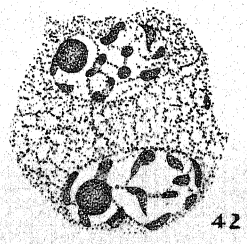
PASTRANA: SPOROGENESIS IN BEGONIA

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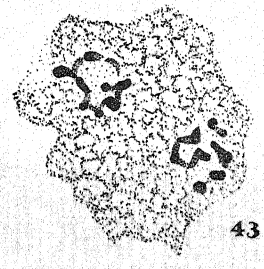
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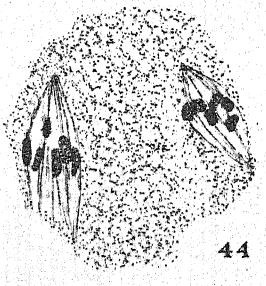
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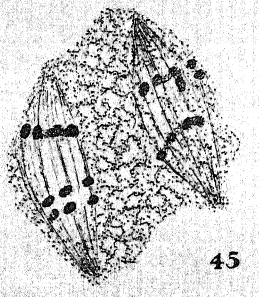
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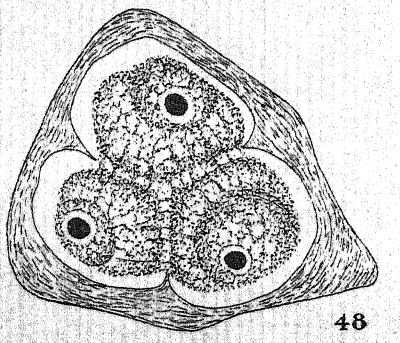
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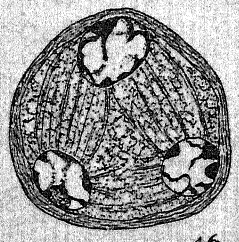
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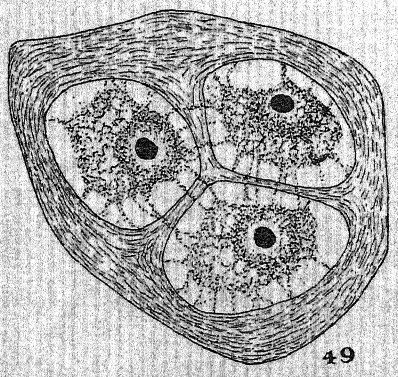
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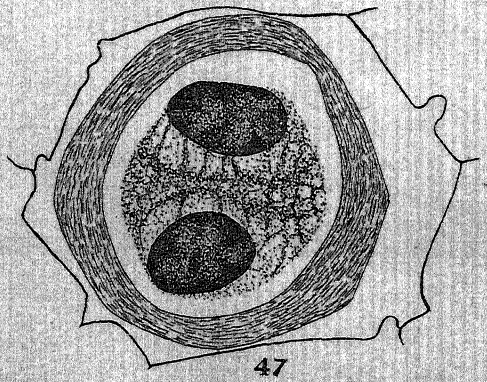
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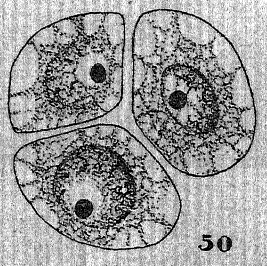
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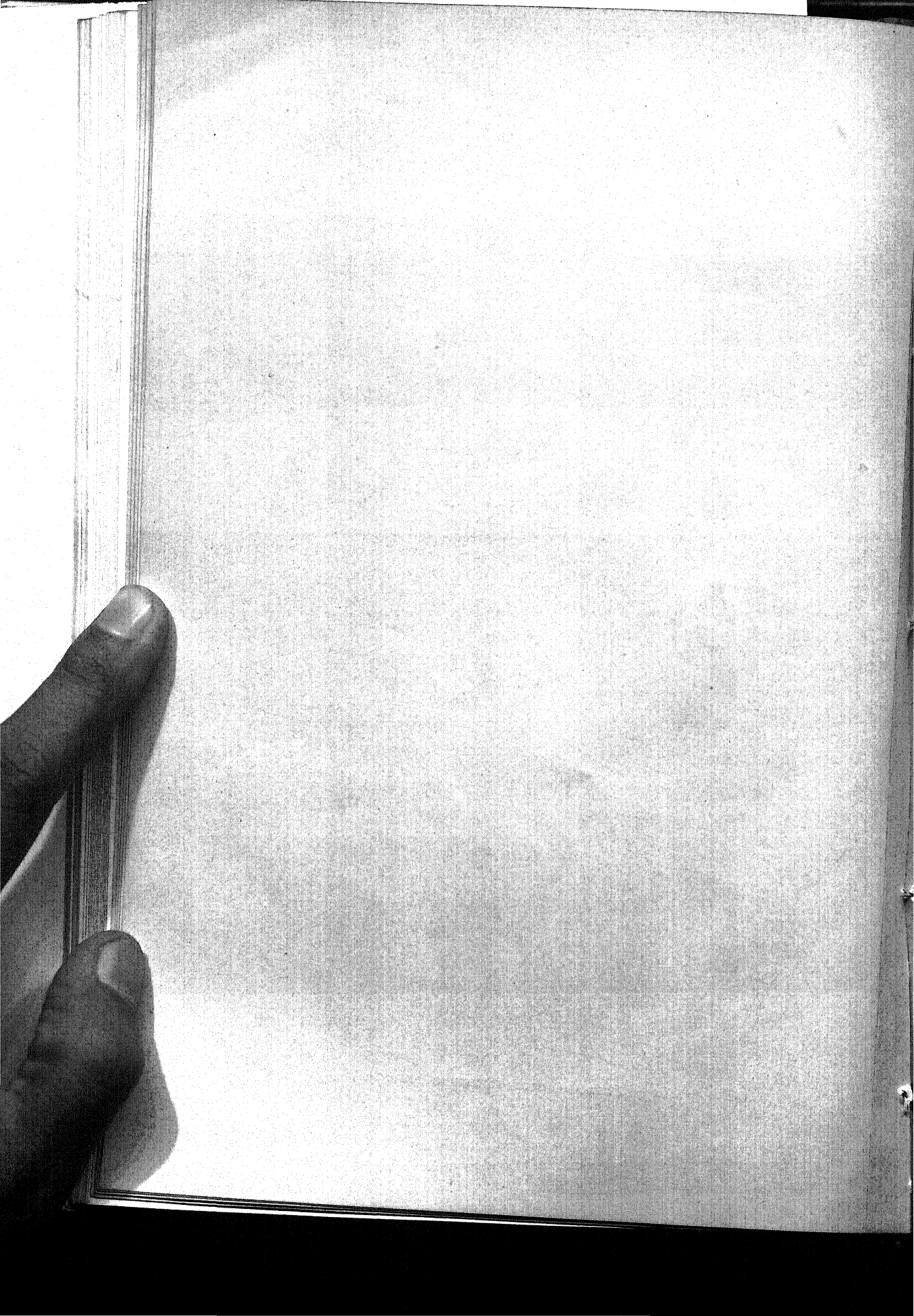
PASTRANA: SPOROGENESIS IN BEGONIA



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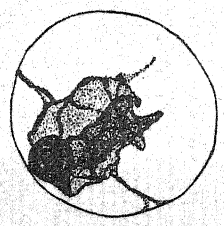

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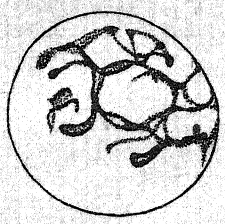

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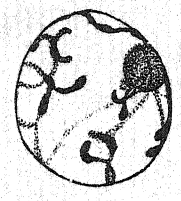

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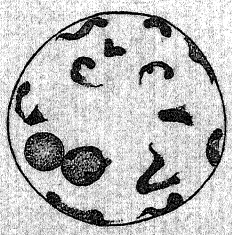

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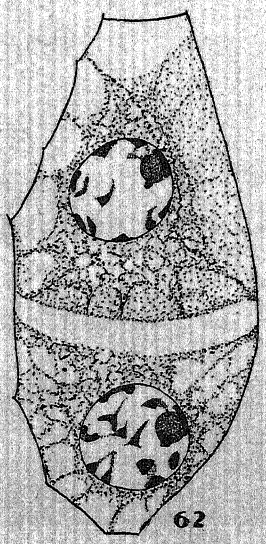

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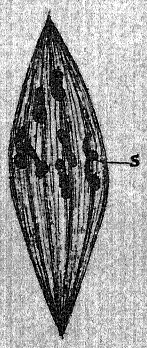

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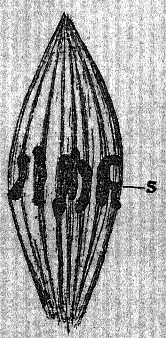

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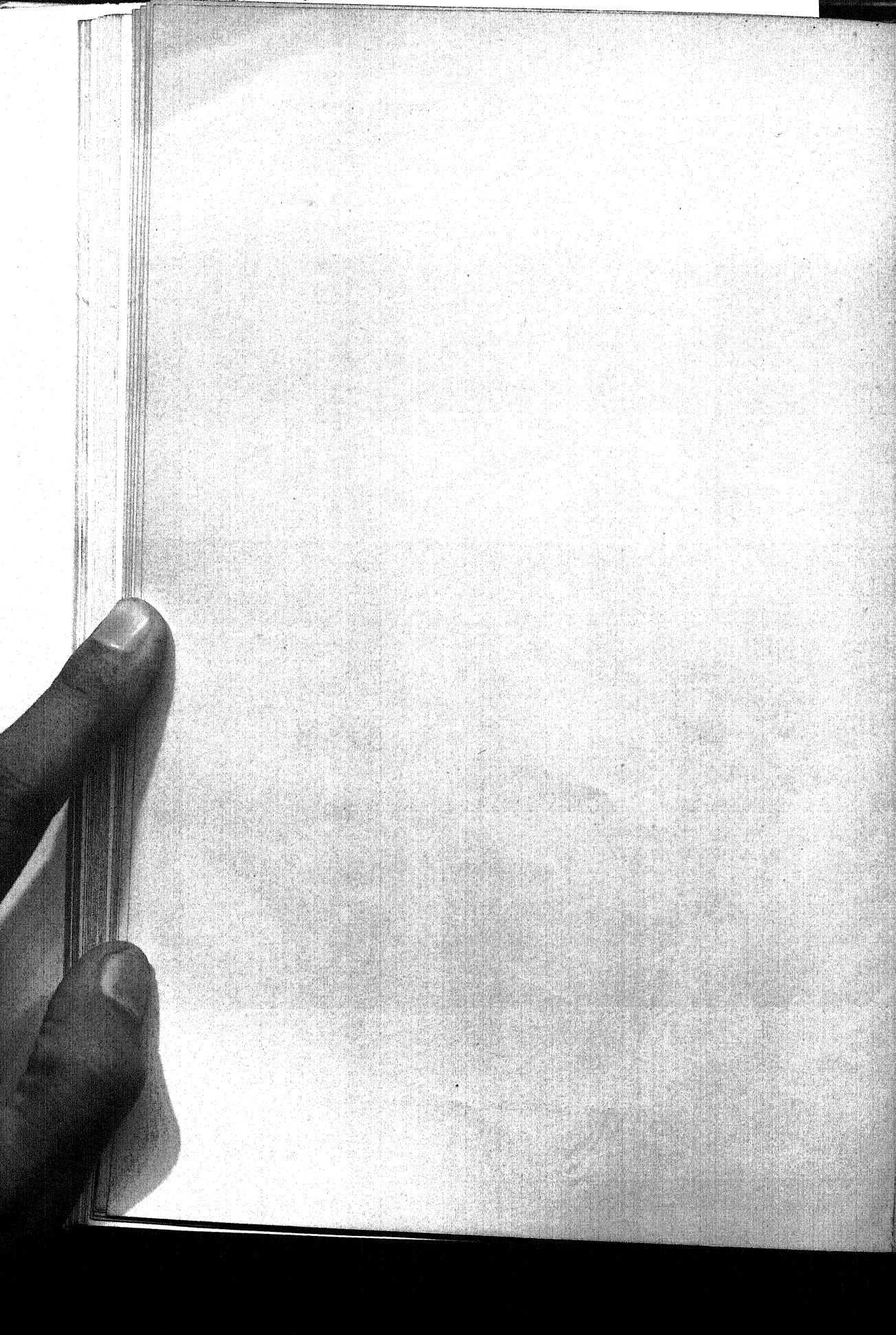

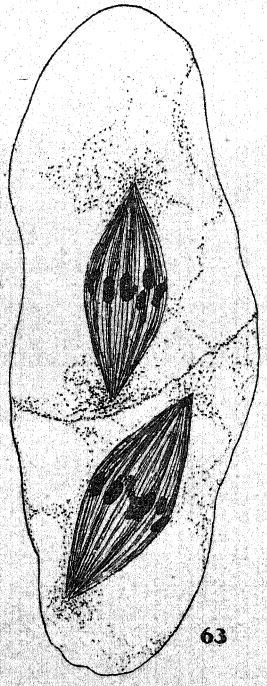

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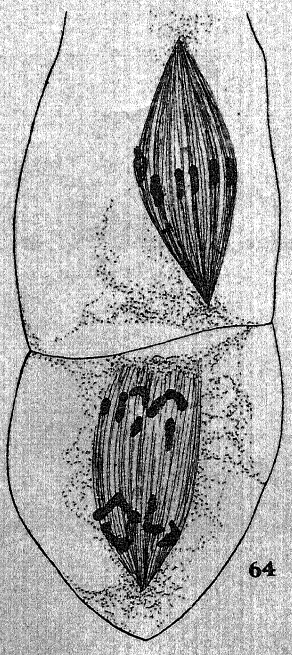

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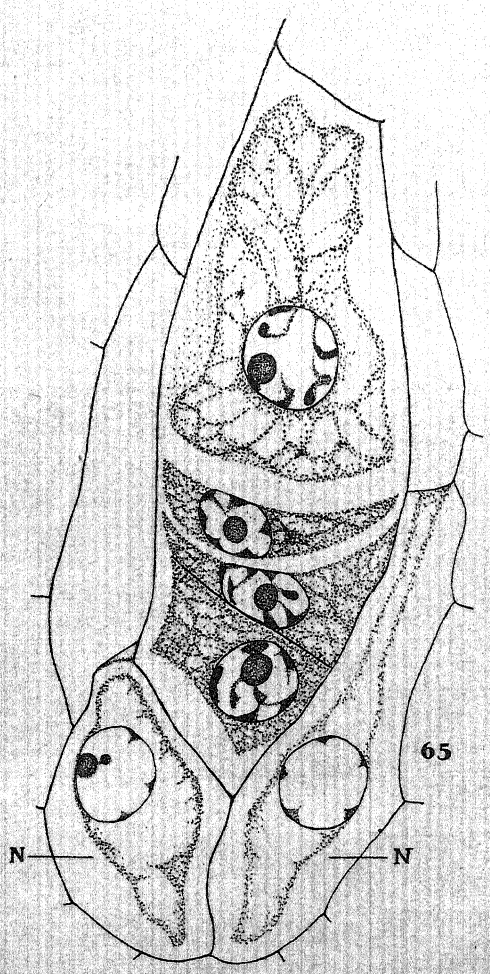

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STUDIES ON *PETUNIA* III. A REDESCRIPTION AND ADDITIONAL DISCUSSION OF CERTAIN SPECIES OF *PETUNIA*

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If the studies of the geneticist are to be of unquestioned value, they must be based on clearly defined material. Organisms used in deriving hybrids for the purpose of genetical analysis should be known to meet our tests, at present not altogether satisfactory, for pure lines. The gametic purity of parents should be determined for at least those characteristics which are to be studied in the succeeding generations. Research, based on a race of animals or plants of unknown descent or on hybrids artificially derived from parents whose genotypical constitution for the characters scrutinized is unknown, can at best not be of the highest value. The above may seem a mere recital of truisms and therefore unnecessary of statement. But one cannot read any considerable portion of the genetical literature which has appeared during the last quarter of a century without feeling the necessity of reemphasizing these fundamental facts. This is especially pertinent to many investigations based on the genus *Petunia*. Due to the looseness with which specific entities have been respected in this genus, it has seemed desirable to redescribe and illustrate the three species of *Petunia* which, in addition to certain cultivated strains, are being studied at Wellesley College. It will thus be perfectly clear what the entities are that form the basis of our investigations. To date only preliminary papers based on these studies have appeared (Ferguson, 1928; Brooks, Watt, and Ferguson, 1930).

Between 1823 and 1835 seeds of *Petunia nyctaginiflora* Juss. and *P. violacea* Lindl. were sent from South America to various European countries. Plants from these were crossed and resulted in a great variety of hybrids, some of them of outstanding value to the horticulturist. A little later Sir William Hooker wrote (1837): "These varieties of *Petunia* . . . were decidedly among the greatest ornaments of the greenhouse in the Glasgow Botanic Garden during the month of May (1836)." With regard to these two species Naudin (1858), as translated from the French, says: "They are

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not two varieties of the same species for when they are completely isolated their seeds reproduce them with perfect constancy and, what is decisive, their hybrids have no constancy." The F_1 generation of the cross between these two species is said to have borne self-colored flowers of varying shades but never darker than *P. violacea* Lindl. In other respects the plants were considered to be more or less intermediate between the two parents. Evidently because of color, it early became the practise of the horticulturists to speak of them as *Petunia violacea.* In fact, as one reviews the early literature regarding the genus *Petunia,* it becomes apparent that the plant growers of that period used *Petunia violacea* as a sort of "blanket tag" for all small-flowered, colored petunias growing in their greenhouses and gardens. It is not therefore surprising that more recent students of the genus have followed their lead and called the garden hybrids on which they have based their studies either *P. violacea* Lindl. or "a variety of *Petunia violacea.*"

Miss Saunders (1911, 1916) used among other forms "one *violacea* plant (commercial material)." When visiting the renowned gardens of Sutton's, England, during the summer of 1930, we were assured that they had plants of *Petunia violacea.* Upon reaching the plot where they were growing, we found magnificent plants abundantly covered with beautiful, large, velvety, deep-purple flowers, the "violacea" of commerce but entirely unlike the species which bears this name.

Skalińska (1921, 1924, 1927) bases her studies on "einer polymorphen Rasse von *Petunia violacea* Lindl." In the last paper she and Cuchtman wrote (1927, p. 3, 4):

> "Die eine von den beiden Extremen ist eine normale, fast aktinomorphe trichterförmige Blute von *Petunia violacea* von ungefähr 4 cm. im Durchmesser, mit tiefrotvioletter Krone. Die Blumen der anderen extremen Form aber sind deutlich zygomorph mit einem Durchmesser von 1, 5 bis 3 cm. Auch in der Blumenfärbung sehen wir Unterschiede. Das rotviolette Anthokyan, das in der ersten extremen Form die ganze Krone gleichmässig färbt, erscheint hier nur in der Blumenröhre in Form eines Ringes und kleiner Flecken, der obere Teil der Krone aber hat eine helle, fast weisse Farbe. Zwischen den beiden Extremen existiert eine ganze Reihe von Übergangsformen, in denen man immer konstatieren kann, dass je grösser die Blume, desto mehr Pigment sie in ihrer Krone enthält, so dass endlich die Pigmentflecken miteinander zusammenfliessen, um eine gleichförmige Blumenfärbung zu bilden. Beide extreme Blumentypen können auch auf einem Individuum erscheinen; solche Pflanzen erhalten somit das Aussehen von Chimären. Aus Samen der beiden extremen Typen, sowie aller Übergangsformen, bekommt man immer eine Nachkommenschaft, die Vielförmigkeit der Rasse wiederholt. Eine Selektion der Sprosse bei vegetativer Vermehrung hat als Folge eine Beschränkung der Variabilitätsskala (6). Jedoch bei der Vermehrung durch Samen kehrt die volle Variabilitätsamplitude der Rasse schon in der ersten Generation zurück."

From this paragraph alone the conclusion seems inevitable that the study is based, not on a pure species, but on a strain of hybrid plants of unknown parentage. Skalińska had earlier (1921) justified such a use of the specific name by assuming that the range in zygomorphy and color is not the result

of an unstable or heterozygous form but is due rather to the variability of a single gene, the different phenotypes belonging to a single genotype. But can one accept the application of Lindley's species to such a variable form? The flowers of *P. violacea* Lindl., as we know them, have a very characteristic shape and are as constant in size and color as one would expect for any species. We have grown during the past ten years 13 lines of *Petunia* comprising 3 species and 10 cultivated strains. We have repeatedly found almost every conceivable variation in certain lines of hybrids, but great constancy of characters in other cultivated strains as well as in the species.

One needs only to observe the photographs published by Malinowski (1928) to be convinced that he could not have been studying a variegated strain of "*P. violacea* Lindl." His flowers vary much in size, the majority are much larger than are the flowers of that species and are not self-colored but variegated.

The studies of Matsuda (1927) and those of Kostoff (1930, 1931) were based on "the garden *Petunia*." Each author discussed his plants under the name of *Petunia violacea*. The plants used by Matsuda grew in the gardens of the University of California at Berkeley and Kostoff's original material was grown at the Bussey Institution, Harvard University. We have the assurance of botanists from both of these universities that no specimens of the true *P. violacea* Lindl. are, or have been to their knowledge, growing in their gardens or greenhouses. At both institutions, however, small-flowered, single petunias of unknown origin have been grown.

The long, cylindrical tubes of the flowers, shown in the photograph, figure 2 *C* of Henderson's (1931) recent paper, are prima facie evidence that the host of his "tobacco ring spot" was not "*Petunia violacea* Lindl." as he states.

The binomial *Petunia nyctaginiflora* Juss. has been less frequently, so far as we are conversant with the literature, misapplied. As early as 1825 Sims described and figured a plant which he called "*Petunia nyctaginiflora*. Large-flowered Petunia." Neither his description nor his colored figure is applicable to the species.

Curtel (1898) described and figured flowers of *Petunia nyctaginiflora* as grown in shade and in sunshine. After growing this species in full sunshine for several summers, we cannot accept his figure 13, Plate XIII, as illustrating the flowers of *P. nyctaginiflora* Juss. The proportion between spread of limb and length of tube is very far from that of the species and is again suggestive of a cultivated form of the white petunia.

The above is not a complete list of citations relative to the point under consideration. But the references are sufficient to make it clear that there should be a more discriminating usage of terms as applied to the species and to the hybrids of *Petunia*. In our studies we have considered a cultivated strain pure for a given character when it has bred true for that character (1) throughout the entire progeny for each of 4 consecutive generations, (2) in

reciprocal crosses between the original parent and later generations, and (3) when individuals from different generations have been crossed, as the 4th generation of the strain with the 2nd generation, etc. When seeds of a species have been obtained the strain is accepted as uncontaminated if for three consecutive generations, beginning with that from the original seed, no aberrant forms have appeared. It is of course to be understood that in these preliminary experiments, as in all later cultures, every precaution is taken to exclude incidental selfing or crossing through natural means. During the fall, winter and spring our plants are grown in a greenhouse supplied with 1000-watt, type C lamps with special reflectors. These lamps are used on cloudy or stormy days and in the early evening. We plan to give the plants from 11 to 12 hours of light a day while in the greenhouse. It is our purpose to keep the houses in which the petunias are growing at a temperature not lower than 50° F. at night or higher than 70° in the daytime, but on hot sunny days the houses of course reach a higher temperature. During the first week in June young seedlings and cuttings of older plants are transferred to the genetics garden. This is a well cultivated field with medium rich soil and full exposure to sunlight. It is enclosed by a "boy proof" fence and fully supplied with a Skinner irrigation system. The study continues in the open field until about September 10, sometimes a little earlier or a little later depending on the season. During the present summer (1931) something over 24,000 pedigreed plants of *Petunia* are growing in this field.

We have constantly endeavored, as partially reported by the senior author (1928), to secure seeds of as many species of *Petunia* as possible. To date we have succeeded in doing so for only three species. Fortunately, these are very important species historically since the generic name was based on two of them, and one of these two and the third are the two species from which practically all the cultivated strains of *Petunia* grown today have descended as a result of intercrossing. These species are *Petunia axillaris* (Lam.) B.S.P., (*P. nyctaginiflora* Juss.), *P. parviflora* Juss., and *P. violacea* Lindl.

We are greatly indebted to Professor Lorenzo R. Parodi from whom we received, on January 5, 1924, mature capsules, well filled with seeds, of *P. axillaris*. These were received under the name, by which they have been commonly known, of *P. nyctaginiflora* Juss. Professor Parodi collected the seeds in Buenos Aires, Argentina, on December 30, 1923. These seeds were sown in flats and from 200 to 300 seedlings were transferred to the genetics field in the spring. The vegetative and floral parts of these plants were practically uniform throughout the entire progeny. From this date to the present time this species has been kept continuously under cultivation both as seedlings and as cuttings. During these eight years five different generations comprising several thousand plants have been grown and not a single aberrant form has appeared. One is surprised at the constant uniformity of both vegetative and floral parts. This species is known in our cultures as population fourteen (P 14). We are further indebted to Professor Parodi for our population

seven (P 7), *Petunia parviflora* Juss. The seeds were collected by Professor Parodi at "Pto Nuevo, Buenos Aires" on December 8, 1929. The notation "Common in Buenos Aires" was on the packet. More than 200 seeds from the packet were sown in early spring. A large percentage germinated. The plants showed great uniformity in all vegetative and reproductive parts and the species has been continued as for P 14.

Not until the summer of 1930 did we succeed in obtaining *Petunia violacea* Lindl. Packets of seeds marked *Petunia violacea* have been received at different times from various sources. In every case plants grown from this seed showed unquestionable evidence of hybrid nature and all were discarded without further culture. Last summer (August, 1930) we received through the courtesy of Mr. A. D. Cotton, Keeper of the Herbarium and Library at the Royal Horticultural Gardens at Kew, England, several cuttings of *Petunia violacea* Lindl. The plants from which these cuttings came were obtained from seed sent to Kew in 1916 by C. E. R. Rolland, vice consul at Montevideo, Uruguay. These plants have not set seed at Kew and have been continued to the present time by cuttings. Only one of the cuttings received by us lived. From this one, however, we have today twenty-eight healthy plants. Four have been kept in the greenhouse during the summer and the rest are in the open field. They show no significant variations either as to vegetative or to floral parts. We have designated this species population nine (P 9). These plants have a marked tendency to self-sterility but cross freely, especially when used as the pollen parent, with the other strains which we are growing.

During the winter and spring many attempts were made to secure seeds by artificially selfing the flowers of *Petunia violacea*. As a result of numerous selfings but three capsules with viable seeds were formed. One capsule contained 88 seeds, one burst during the night and the next day but 2 seeds remained in it, and the third yielded but 59 seeds. Strong young plants are now growing from these seeds. They have just begun to flower and so far they are not distinguishable from the young cuttings of our original stock. To our surprise this summer several capsules have appeared on the plants in the greenhouse and on those in the open. Because of the isolated position of these plants in the greenhouse, at this time, one feels very strongly that the capsules have formed as the result of natural selfing. However, no definite conclusion as to the method of pollination can be drawn until the plants from these seeds have fully matured. It is significant in this connection that for several months during the winter and spring, when the plants of *P. violacea* were surrounded on all sides by other species and cultivated strains of *Petunia* not a single capsule set on them as the result either of natural crossing or of selfing. It has occurred to us that the apparent self-sterility of *P. violacea* may be a matter of climate. The capsules under consideration came after several weeks of hot dry whether, quite unlike the climate of Kew, England, or the climatic conditions in our greenhouses during the winter and spring of the present year.[1]

[1] Knuth (1899, p. 135) says: "*Petunia violacea* ist nach Darwin selbststeril."

A critical study during the summer of 1930 of the petunias in the herbarium at Kew only served to strengthen our conviction that we have among our cultures the three species named above. Among other interesting sheets observed were several of Tweedie's plants of *P. violacea* and *P. nyctaginiflora* as preserved in Hooker's herbarium. In addition to this, the type locality for the three species which we are growing is the region of the Rio de la Plata. The seeds which gave rise to our populations 7 and 14 were collected in Buenos Aires, or south of the river; and the seeds giving rise to the Kew plants, from which our population 9 was derived, were from Uruguay or north of the river.

In 1803 A. L. Jussieu founded the genus *Petunia* upon plants collected from the mouth of the Plata River and preserved in the Commerson Herbarium. These plants he named *Petunia nyctaginiflora* and *P. parviflora.* One of the species, *P. nyctaginiflora,* had already been described under the name of *Nicotiana axillaris* by Lamarck in 1793. Lamarck's type was also collected by Commerson but from Montevideo, on the north bank of the Plata River. No reference to Lamarck's name for this species was made by Jussieu and this earlier description was doubtless unknown to him. It was not until 1888 that the earliest trivial name, *axillaris,* was associated with the generic name *Petunia.* At this time Britton, Sterns, and Poggenburg listed *Petunia axillaris* (Lam.) in their Preliminary Catalogue of Anthophyta and Pteridophyta. In his study of the Petunias of South America as preserved in various European herbaria, Fries (1911) had the opportunity of seeing a sheet collected by Commerson at Montevideo. As a result of this study he accepts Britton, Sterns, and Poggenburg's decision regarding the synonymy of *Petunia nyctaginiflora* Juss. and *Nicotiana axillaris* Lam.

The following description of our P 14, *Petunia axillaris* (Lam.) B.S.P., is based on plants growing in the open and in full sunshine during the month of August. However, the description would vary scarcely at all if based on plants growing in large pots in a well lighted and ventilated greenhouse in February. Plants grown from seeds or from cuttings have one erect central stem which later becomes declined with an assurgent tip. This central stem is surrounded at the base by a rosette of decumbent branches. From the declining central stem as well as from the rosette of lateral branches erect stems are sent up which may reach a height of from two to three feet, giving the whole plant a "bushy" appearance. The herbage and flowers are markedly viscid-pubescent, the plants being clammy to the touch. In young seedlings and in older plants, especially when not growing under the best conditions, there is often a violet-purple pigment in the stem particularly in the region of the nodes. Leaves from the base of the stem to the first flower on any shoot have an alternate arrangement. The first flower terminates the monopodially developed stem and from this point on a given stem is continued sympodially. Following the first flower the shoot is made up of a series of two-leaved branches each being terminated by a flower. The internode of the short

branch is so reduced that the leaves appear to be opposite (Pl. XXXIII, fig. 1). Both Lamarck and Jussieu speak of the flowers as axillary. In his description of *Nicotiana axillaris* Lamarck wrote "*pedunculis axillaribus*" and Jussieu used the expression "*flores solitarii, axillares.*" But Jussieu's illustration shows the sympodial stem, evidently incorrectly interpreted by him. The trivial name of this species, *axillaris,* is therefore a misnomer. The correct method of branching was described in detail and illustrated diagrammatically by Fries in 1911.

The leaves vary greatly in shape and size. Under favorable conditions the lower leaves may attain a length of 12 cm. or more. They vary from ovate to ovate-lanceolate with long, margined petioles (Pl. XXXIII, fig. 2). As they ascend the stem they become progressively reduced in size to smaller (about 3 cm. long) short-petioled leaves tapering to the base and to an acute apex. They are essentially the same as the leaves on the axillary branch *e* shown near the base of figure 1. When the plants are young the leaves of the floral branches may be 3 cm. or more in length but as the plant ages, or conditions become less favorable for growth, they are gradually reduced in size often attaining scarcely more than 1 cm. in length. These reduced leaves are subsessile and range from ovate to broadly ovate with acute apex.

The white flowers are large, viscid-pubescent on the outside, and are set obliquely in relation to the stem. The flower is distinguished from all other Petunias which we have seen by its long cylindrical tube which widens scarcely at all before reaching the throat. The calyx is deeply parted with the largest of its unequal lobes two or more times the length of its tube. The segments are never broad and range from lanceolate to linear with an acute apex. In general there are two long segments and three short ones; the longer segments vary from 11 to 15 mm. and the shorter ones from 9 to 12 mm. in length. The calyx-tube is around 6 mm. in length. The corolla is subsalverform with the limb only slightly zygomorphic. As an average of several measurements of flowers taken at random, but of the same age, the greatest diameter of the limb is 49 mm., the length of tube and throat 58 to 60 mm. The throat is very short, being not more than 1/6 the combined length of tube and throat (Pl. XXXIII, figs. 1*c*, 3). The white limb is sometimes very faintly tinted with pale hortense violet along the midveins especially near their outer ends. The background color of the throat is pale turtle green; this is marked with a reticulum of dark perilla purple.[2] This special marking of the throat is strongest on the posterior petal and fades out entirely on reaching the midveins of the two anterior petals (Pl. XXXIII, fig. 3). The first two cm. of the tube, viewed from without, are naphthalene violet but as the tube gradually increases in size the veins, along which the pigment is distributed, are separated by a greater distance and the color is therefore lighter. The

[2] All colors ascribed to flower or floral parts are based on Ridgway's Color Standards and Color Nomenclature. The matching is always done, in a darkened room, with a special daylight lamp.

violet pigment is markedly continued on the under side of the limb throughout the entire length of the midvein of each petal and may extend slightly into some of the secondary veins, often giving the exterior of the limb a suggestion of very pale hortense violet. The filaments are erect and the stamens are very slightly if at all didynamous. The height of the anthers varies somewhat, but in general four of the five stamens have their anthers nearly at the same level and standing just beneath the stigma. The fifth anther lies slightly below the others (figs. 3, 4). The filaments of the four longer stamens are adnate to the corolla to a point just above the middle of the tube. The shortest stamen stands between the two anterior petals and has its filament joined to the tube for a varying distance above the others, rarely not becoming free until just below its anther. The innate attachment occurs at a point just below the middle of the anther. The anthers and pollen are pinard yellow. The pollen grains are bilateral and very uniform in shape and size. The most characteristic length of grains is 54 μ. Of the 500 grains measured (100 from each of 5 plants) the range was from 47.8 to 64.8 μ. While both the upper and the lower limit occurred but once, with scattering numbers at the points between, 122 grains were 54 μ long. In full grown flowers the pistil is from 60 to 65 mm. long with the green ovary only about 6 mm. in length; the long filiform style is slightly bent about 3 mm. above its point of attachment (Pl. XXXIII, fig. 5). The placentation is axile with the two placentae very broad. The stigma stands in the upper half of the throat; it is discoid and has a liplike projection on either side formed by the recurving of the carpels at these points; the point of union of the two carpels is indicated by a darker line giving the effect of a slightly bilobed stigma (figs. 4–7). The stigma is turtle green, the style citron green.

The species is very self-fertile and fruits abundantly both in the greenhouse and in the open. In general more than 95 percent of the flowers form large capsules. The fruit is asymmetrical and bivalvular; the posterior valve is the larger and curves over at the apex, suggesting, when partially dehisced, the open beak of a bird. A given capsule may have from 800 to 1000 or more seeds. As the fruit matures the peduncle remains erect and elongates considerably, and the calyx increases in size. As the capsule loses its green color the persistent calyx-lobes become reflexed (Pl. XXXIII, figs. 1*a*, 8, 9). The dull straw-colored capsule varies from 1 to 1.5 cm. in length. It dehisces septicidally into two valves. The base of the style is persistent and in drying forms an irregular ring over the apex of the capsule. With age this may be sloughed off and the valves eventually become more or less bifid (fig. 9). The numerous seeds are small, asymmetrically oval with the end opposite the hilum somewhat truncate. They are brown in color and foveolate, the edges of the foveoli being delicately incised (fig. 10).

We have every reason for believing that our plants, as described above, are true representatives of the species *Petunia axillaris*. That the plants from which Professor Parodi collected the seeds had not been contaminated as a

result of natural consorting with other species is evidenced not only by their behavior in successive generations but also by the character of their pollen grains.

Miers (1846) gave the name *Petunia propinqua* to a South American white-flowered petunia which he suggested might be only a variety of *Petunia nyctaginiflora.* He describes it as differing from the latter species in being somewhat prostrate and less pubescent; the leaves narrower; the floral leaves petioled and the calyx-segments nonfoliaceous. The corolla is described as having five purple lines. Sendtner (1846) considered Miers's species a variety of *P. nyctaginiflora* Juss. and Fries (1911) included it under *P. axillaris* (Lam.) B.S.P. In addition to the descriptions of white-flowered petunias by Miers, Sendtner, and Fries, which were based on herbarium specimens, is the description of Sims (1825) under the name *Petunia nyctaginiflora,* of a short-tubed, white-flowered form growing in the Chelsea botanical garden. He not only states that the corolla is marked with green veins and that the stigma is two-lobed but clearly shows these characteristics, together with the short tube, in his illustration. Because of the close similarity of Sims' plant to a strain which we are growing, described below, it does not seem to be a short-tubed form of *P. axillaris,* but rather a hybrid into which that species had entered.

In 1919 we secured seeds of a " single bedding petunia—white " of R. and J. Farquhar Co., Boston. This has been inbred for ten generations and the individuals of the last generation are strong, vigorous plants in no way different from the original stock. They have bred true for habit, foliage and floral parts. This strain is known in our cultures as population six (P 6). The general habit of the plant is similar to *P. axillaris* and it has been spoken of by some who have seen it growing as " a short-tubed form " of that species. But there are marked and constant differences. There is never any purple or violet in its stem or in the limb of its corolla. The calyx-lobes are slightly shorter and more uniform in size for a given flower; there is a more evident enlargement of the corolla-tube at the point where the filaments become free and the combined tube and throat is always 1 to 2 cm. shorter, and the limb somewhat broader than in the species. The corolla is always a beautiful clear white with a faint touch of green along the midveins and the naphthalene violet associated with the veins on the exterior of the flower in *P. axillaris* is constantly replaced by javel green. The colors of throat, anthers and pollen very closely resemble those of *P. axillaris* except that there is slightly more green and less purple in the throat. Four stamens are distinctly didynamous with the anther of the fifth standing just below the lower pair of anthers; the filaments are erect but are distinctly, though slightly, arched inward at the point where they become free from the corolla-tube; as a rule all 5 stamens are adnate to the tube for practically the same height, but the shortest one may be adnate for 2 or more mm. higher than the others and one stamen, not always the shortest, may be adnate throughout the entire length of its filament.

The pollen grains are pinard yellow. They are shorter and there is a larger number, not excessive, of abortive or irregular grains. In length they conform more nearly to the grains of *P. violacea* than to those of *P. axillaris* but they have a greater range than either species and practically the same number of grains fall at several points between the extremes. There is thus, within certain limits, no one length more characteristic than another. The turtle green stigma is like that of P 14 except that it is slightly larger and much more distinctly bilobed; it overtops the anthers and as the result of a slight curve in the upper part of the style it faces somewhat towards the posterior lobe of the corolla. It does not fruit abundantly; less than 10 percent of the flowers, when growing in the open, set seed but a larger proportion of artificial selfings are successful. The capsules are rarely large; the two valves are equal in size and the apex is slightly if at all recurved. The largest number of seeds counted from a single capsule is 461, but ordinarily the number is less. There is not as marked an increase in size of the calyx-lobes and in length of the peduncle during the maturation of the fruit as in the natural species. Very rarely one finds a flower with 6 corolla-lobes or one with a petaloid stamen.

The differences between P 14 and P 6 as listed above are so many and so clean-cut as to make it clear to us that our cultivated white petunia is not "a short-tube form" of *P. axillaris* but a hybrid which has become homozygous for many of its characteristics. This conviction is further strengthened by the fact that in hybrids of these two strains we never get a series of intergrades. We have growing in the field this summer (1931) 980 hybrids of P 6 × P 14 and P 14 × P 6. Vegetatively they have the same habit as the parents but are even more vigorous than either. The leaves are larger and more ovate. The flowers are intermediate in practically all details. There is some violet pigment associated with the veins on the outside of the corolla-tube and with the midveins of the limb at the time of anthesis, but when the flower is fully opened only the green of P 6 remains. From about 20 to 80 percent of the flowers, depending on the pollen parent, set fruit with viable seeds, the capsules often being as large as those of *P. axillaris,* but with the two valves more nearly equal and the "beak" less arched. It is very interesting to note that the plants, which result when *P. axillaris* is the pollen parent, are much more fertile, the capsules larger and the seeds more numerous than when P 6 is the pollen parent. This is doubtless attributable in some measure at least to the variations in the character of the pollen grains of the two populations as already described. In this connection we would call attention to the rather unique sterility of *P. axillaris* as outlined in an earlier paper (Brooks, Walsh, and Ferguson, 1930). Briefly, its pollen is very active on its own stigma and on that of its sibs; but as a rule no seeds are formed when its pollen is applied to the stigma of the other strains of *Petunia* which we are culturing. In the paper referred to, one exception, in which a few seeds were occasionally formed, was noted. That capsules should develop containing several hundred seeds when its pollen grains are placed upon the

stigma of P 6 suggests, at least, that the relationship between this pure-breeding, cultivated white and the wild species, as regards certain significant factors, is still very close.

There is no doubt in our minds but that this strain is of hybrid origin, but for twelve years it has been so consistent in behavior both when selfed and when mated with other strains or species that we believe it has attained the dignity of a horticultural species and assign to it the name *Petunia alba* Hort.

No question is likely to occur regarding the identity of our population seven (P 7)—*Petunia parviflora* Juss. The plants are prostrate and creeping forming mats from two to four feet in diameter. It is an excellent example of a "carpet-plant." The stems hug the ground closely frequently taking root at the nodes (Pl. XXXIV, fig. 11). At no point does it rise more than an inch above the ground. The short-petioled, slightly succulent leaves range from linear to linear-spatulate with both surfaces and the margin set with minute capitate hairs. The viscid nature of the herbage is scarcely noticeable to the touch. The mature leaves vary from 1 to 2.5 cm. in length. As in *P. axillaris* the stem becomes sympodial at the point where a flower occurs. But in this species, the flowering and the purely vegetative parts of the plant are not distinctly separated. That is, a short monopodial stem with one or more alternate leaves may be inserted between flower-bearing portions of the stem. The leaves associated with the flowers are similar in shape to those on the vegetative portions of the stem. This is in marked contrast to *P. axillaris* where the floral leaves are always distinctive and the sympodial stem, once established, continues until the growth of that particular shoot ceases.

The flowers are very small in comparison with those of the other two species under consideration and are short-lived. In the vicinity of Wellesley they open about eleven o'clock in the morning, begin to close about three in the afternoon and do not open again. In young plants the calyx-lobes ordinarily exceed the corolla in length but in flowers of the same plants, at a somewhat later date, they rarely reach the upper limit of the throat. Both conditions may occur on mature plants at the same time, but as the plants age only flowers with the shorter calyx are as a rule formed (Pl. XXXIV, figs. 12, 13). The calyx-tube is scarcely more than 1 mm. in length and its lobes are very like the leaves. The corolla consists of a short cylindrical tube, a longer, ventricose, funnel-form throat and a small subrotate limb. The limb is very slightly zygomorphic. A transverse fold, opening outward, occurs near the base of the limb of the two anterior petals (figs. 12*a*, 13*a*, 14). On vigorous young plants the length of the tube and throat varies from 6 to 7 mm., the tube being less than 1/3 of the combined length of the two. The limb, at the moment of greatest expansion, is from 5 to 7 mm. in diameter. It is a light mallow purple; this shades into the yellow throat and the yellow becomes lighter as it passes into the tube.

Four stamens are didynamous. Their filaments and the style all curve

abruptly near the top so that the anthers and the stigma face the posterior petal (figs. 15, 16). The fifth stamen is anterior and much shorter. The filaments of the didynamous stamens are adnate to the tube for about 1 mm. and the filament of the anterior one for about 2 mm. The anther attachment is innate. The color of the anthers is pinard yellow, of the pollen empire yellow. The pollen grains are bilateral and range in length from 38.24 to 30.60 μ, with the most commonly occurring length 36 μ. In measuring 500 grains, 6 reached the highest limit, 1 the lowest limit and 127 were 36 μ in length, or 18 μ shorter than the most commonly prevailing length in *P. axillaris*. The stigma is narrowly elliptical and slightly recurved at both ends (figs. 17–20). Self-pollination takes place slightly before or at the time of anthesis and the plants fruit abundantly. During the fruiting period, the peduncle elongates but little and ordinarily maintains an acute angle with the stem but occasionally it may become recurved (fig. 11 *a, b*). The fruit does not therefore have a uniform position at maturity as in *P. axillaris* and *P. violacea*. The 5 to 6 mm. long capsule is conical and symmetrical, the two valves being equal. The dehiscence contrary to that of the other species is septifragal. The calyx remains green and leaflike until the yellowish brown capsule has dehisced (fig. 21). A given fruit produces a hundred, more or less, seeds which are smaller than but otherwise similar to the seeds of *P. axillaris* (fig. 22).

Of the three species being considered *Petunia violacea* Lindl. offers by far the most difficult problem as regards identity. The three binomials, *Salpiglossis integrifolia* Hook., *Nierembergia phoenicia* D. Don, and *Petunia violacea* Lindl., published in England from 1831 to 1833 have been considered by many botanists as representing the same species. *Petunia violacea* is the combination that has been in general use in taxonomic, horticultural and genetical literature. An examination of much of this literature with the illustrations makes it clearly evident that the forms to which the name *P. violacea* has been applied present a distinctly heterogeneous assemblage. The early descriptions leave much to be desired so that one cannot be certain as to whether *violacea* is or is not the earliest trivial name applied to this species. Small wonder that more recent students have added to the confusion.

It is the general belief today that the garden petunias have arisen as a result of crossings between a white-flowered and a purple-flowered species of the South American genus *Petunia*. *P. axillaris* and *P. violacea* are accepted as the two species involved, the former being the white ancestor and the latter the colored one. As previously stated, the types for Lamarck's *Nicotiana axillaris* and for Jussieu's *Petunia nyctaginiflora* were collected in South America and unquestionably they represent one of the wild species that entered into the constitution of the cultivated petunias. Unfortunately what is considered to be the purple-flowered ancestor was first described and named, not from plants grown in South America, but either from plants, or their seedlings, grown in Scotland from seeds originally collected in South America or from plants growing in England from seeds the origin of which is unknown.

It is possible that the seeds came from South America, but there appears to be a strong probability that the plants described for this species represent natural hybrids between *P. axillaris* and the South American colored ancestor of the garden petunias. The reasons on which this assumption is based are given later. It must be determined if possible whether or not Lindley's plant represents the colored parent and if not what is the entity that does. If Lindley's is the species was he correct in his citation of synonyms?

The following description of *Petunia violacea* Lindl. is based on plants grown from our Kew cuttings and on young seedlings derived from them by artificial selfings. The main stem may remain erect until 8 or 9 inches high and is early surrounded at the base by several lateral branches. All the stems are slender and weak and very soon become reclining with assurgent tips. The branches interweave closely covering the ground and produce an abundance of brightly colored flowers. The herbage is viscid-pubescent but only slightly clammy to the touch. A stem is monopodial until terminated by a flower; following the first flower an axis is continued sympodially as described for *P. axillaris* (Pl. XXXIV, fig. 23). The leaves below the flowering part are elliptical-ovate or elliptical-lanceolate and have a longer or shorter margined petiole. The largest leaf observed was on a seedling plant and had a length of 9.4 cm. with a width of 3.4 cm. Commonly the proportions are about 3 to 4 cm. long by 1.5 cm. wide. The leaves following the first flower are in general of the same shape and size as are those below the flowering portion; but as the season advances they are gradually reduced in size and the flower-bearing portions may come to resemble an inflorescence, the leaves becoming almost bractlike (fig. 24).

The flower is much shorter than that of *P. axillaris,* being in general from 3.2 to 3.6 cm. long, and has a very characteristic shape (figs. 23 and 25). The calyx-tube is about 2 mm. long and its five unequal segments are strap-shaped. In a given flower they vary by 4 to 6 mm. in length, with the largest segment rarely more than 21 mm. long. The brightly colored campanulate corolla is distinctly zygomorphic with the posterior petal bent back almost to a right angle with the throat. The broad, ventricose, funnel-formed throat is about 13 mm. long; the tube is much shorter being in general about 5 mm. in length (fig. 25). The color of the limb is between mallow purple and tyrian pink with a deeper purple along the midveins; the throat has a background-color of phlox purple. The veins of the throat are Rood's violet but this color gradually fades out as the middle of the anterior petals is approached. The five stamens are adnate to the tube throughout its length; four are didynamous; the anthers of the longer pair meet above the stigma while those of the shorter pair come into contact directly beneath it; the fifth and anterior stamen is shortest and lies between the two members of the longer pair. By a curve in the upper part of the filaments the anthers face the posterior petal (figs. 26, 27). After dehiscence the two lobes of an anther become divaricate at the base forming a *V*-shaped body (fig. 26); they are pale Russian blue, the

pollen cadet gray. The pollen grains are midway in size between those of the two species described earlier. They range from 38.70 to 49.50 μ; there was only 1 at the lower limit, 4 at the upper, with the largest number (111) of the 500 measured 45 μ long, or just 9 μ shorter than the most characteristic length for *P. axillaris* and 9 μ longer than that for *P. parviflora*. The style is slightly angled in its upper portion and a short distance below the stigma it is bent to an angle of about 45 degrees thus causing the stigma to face the posterior petal. The color of this portion of the style is perilla purple. The stigma is four angled, and naphthalene violet in color. The disc at the base of the ovary is more prominent than in the other two species (figs. 28–31).

Although two stamens have their anthers directly above the stigma and therefore offer a most favorable condition for self-pollination, the plants are markedly self-sterile, setting fruit only occasionally. As the fruit develops, the calyx segments continue to grow and come to stand more or less at right angles to the enlarging capsule (Pl. XXXIV, fig. 32); the peduncle turns back towards the stem and is strongly reflexed before the fruit is mature (figs. 23*a*, 24*b*, *c*). The broadly conical, symmetrical capsule is bivalvular and dehisces septicidally. The seeds are comparatively few, 88 being the largest number yet counted in a given capsule; they are of the same general type as described for the other two species (fig. 33).

We find no reason to doubt that our plants as described above, are representatives of the same species as are the Tweedie plants of *Petunia violacea* which are preserved in the herbarium of the Royal Botanic Gardens at Kew.

In 1831 W. J. Hooker published a description and colored illustrations of *Salpiglossis integrifolia*. He writes: "This new and most distinct species of *Salpiglossis* was raised from seeds sent in the autumn of 1830, by Mr. Tweedie of Buenos Aires, to the Glasgow Botanic Garden, where the young plants, placed in a warm exposure in the open air produced their richly coloured blossoms in July of the following year (1831)."

In Sweet's British Flower Garden for June 1833 there appeared a description and colored plate, taken from the manuscript of D. Don, of *Nierembergia phoenicia*. After describing his plant, Don says: "It is a native of the countries of the Rio de la Plata, but is found to succeed uncommonly well in the open border of this country." He cites Hooker's *Salpiglossis integrifolia* as a synonym of his *Nierembergia phoenicia*.

A few months later, November 1833, Lindley published in the Botanical Register a description and colored plate of a plant which he named *Petunia violacea*. He gives as synonyms *Petunia violacea*. Brit. fl. gard. n.s. 193;[3] *Salpiglossis integrifolia*. Hooker in Bot. Mag. t. 3113; *Nierembergia phoenicia*. Brit. fl. gard. n.s. 193. Lindley does not indicate the source of his material but states that the plants are native of Buenos Aires and speaks of their utility for the garden.

[3] No reference to *Petunia violacea* occurs under 193 of Sweet's British Flower Garden. The name used here is *Nierembergia phoenicia*.

A consideration of the texts and illustrations of these three authors leaves one very uncertain as to whether or not they were studying individuals of the same species. The vegetative portions shown in Hooker's plate are very like those of *Petunia violacea* as we have come to know it; but the flowers as illustrated are not those of the species under consideration. They have a distinctly rotate limb and the throat is much lighter than the limb. This is disturbing for Hooker makes the statement: "the tube dark bluish purple." Neither the stigma nor the stamens as figured and described correspond to those of *violacea*. We did not find Hooker's type plant among the species of *Petunia* preserved at Kew.

Of the three early descriptions Don's is by far the most explicit. But many floral characteristics which he lists are not true for our *P. violacea*. He shows only one leaf on the stem near the origin of each of the first two flowers and says: "Flowers oftentimes seated opposite the leaves." The leaves are much too broad and too nearly sessile for the species. He writes: "It is readily increased both by seeds and cuttings; but young plants raised by the former mode are to be preferred, the blossoms in them being in general much larger, as in our specimen, which was derived from a seedling plant, communicated by Mr. Neill to Mr. Lambert, and which flowered at Boyton last month." From this and other statements in his text one must conclude that Don's type was grown in a nursery and the indirect evidence points to its hybrid nature.

Lindley's description is very brief and not diagnostic. While his illustrations more nearly resemble our plants of *P. violacea* than do those of Hooker or of Don, he does not show the characteristic flower shape and the leaves are very broad in proportion to their length. Both his flowers and leaves are more suggestive of the F_1 hybrids, in our cultures, than of the species. It also seems probable from his text that he was describing a natural seedling from the garden. He writes: "There is perhaps no plant yet in our Gardens which is quite so handsome as this." . . . "Planted singly against a wall or fence in the open ground, it is also a striking ornament; but it is only when collected into a large bed in the open air, and allowed to entangle and interlace its branches, till every piece of soil is concealed, that its whole splendour is developed. It then produces, from August to October, a succession of its purple blossoms, which are yielded in such profusion, and of so bright a colour, as to render the spot where it grows almost too bright to look upon." . . . "But from *Petunia,* of which the now common *Petunia nyctaginiflora* may be taken as the type, the plant differs in nothing whatever except the inflated tube of its corolla and the size of its embryo." As suggested by Bailey (1898) this comparison could not possibly have been made if he were describing the species *Petunia violacea*. Moreover this makes it clear that *P. axillaris* was commonly grown in the same gardens with *P. violacea*. As a result of this association natural hybrids were doubtless freely formed. There is abundant evidence of such crossing in the literature.

The flowers of Hooker's illustration together with his description fail to convince one of the exact identity of his plant. The flowers do not resemble those of *P. violacea* or of any *Petunia* known to us. But assuming they are *Petunia* the likeness is not sufficiently clear to warrant a change of combination. If both Don's and Lindley's plants were hybrids, as seems probable, there is no point in changing from Lindley's name to Don's on the score of priority. But however strong one may believe the indirect evidence to be, there is now no way of proving that Lindley's plants were not the direct descendants of seeds secured from South America. We, therefore, advocate keeping the well known name—*Petunia violacea* Lindl.

In so far as we know the first description of the species under consideration, based on a study of plants which actually grew in South America, is that of Sendtner (1846). His description was based on material collected by Sellow in southern Brazil. He accepts the binomial *P. violacea* Lindl.

Fries (1911) also retains Lindley's name for the species. It is not clear to him to what species Hooker's plant belongs. He finds nothing like it in the material which he has seen. He, therefore, does not change the combination to *Petunia integrifolia.* He had no opportunity to consult the description by Don as published in Sweet's British Flower Garden and hence does not feel justified in discarding Lindley's combination on the basis of priority. Fries' descriptions and illustrations are based on herbarium material. The very closely allied species, *P. inflata* R. E. Fries and *P. occidentalis* R. E. Fries, may be separated from *P. violacea* Lindl. according to Fries, on the basis of the position of the fruit, which is recurved in the last named species and erect in the other two.

In connection with the early descriptions of *Salpiglossis integrifolia, Nierembergia phoenicia* and *Petunia violacea,* with their flowers much smaller than those of the ordinary garden petunias, it is interesting to consider a small-flowered plant which occurred as the result of self-sowing in our genetics field in 1929, two years before *P. violacea* was growing in this region or in fact in this country. In this field plants of *P. axillaris,* several strains of garden petunias, and various hybrid petunias were growing. The new form has been preserved to the present time by means of cuttings. It is known in our cultures as population ten (P 10). This summer (1931) capsules were observed on the plants for the first time. The aspect of the plant is in general much like that of *P. violacea.* The most evident differences are that the stems are somewhat more erect, the leaves slightly wider and more ovate. The peduncle is longer varying from 3 to 7 cm. in length and does not recurve as the fruit matures.

The diameter of the throat is somewhat less, otherwise the flowers are almost identical in shape and size with those of *P. violacea.* The color of the corolla is deeper being redder and brighter than rhodamine purple. The background of the throat in young flowers is light phlox purple with the veining on the posterior side prune purple; in older flowers the throat is

lighter in color than the limb, the background becoming phlox pink. The color of the style from the point of bending to the stigma is light perilla purple. The stigma is purplish lilac in color and in shape is unlike that of any other *Petunia* which we have seen; it approaches an ellipse in form and has a papillose border or rim (Pl. XXXIV, figs. 34–37). Even with the differences noted this plant might easily be taken for *P. violacea* unless one were very familiar with the species. When petunias escape from cultivation there is evidently a tendency to revert to forms resembling the colored-flowered ancestor of the cultivated strains. It is very probable that such segregates have frequently been described as *P. violacea.*

The plant of "*Petunia violacea*" described in Paxton's Magazine of Botany (1834) is unquestionably a hybrid between *P. violacea* and *P. axillaris.* The limb of the corolla is too large, the purple too dark and the leaves more ovate than for *P. violacea.* Paxton does not give the origin of the plant he figures but speaks of beds planted with *P. violacea* "at Chatsworth last summer." Mrs. Loudon (1840) uses the binomial *Petunia violacea* but to her figure 2 of the accompanying colored plate she gives the name *Petunia phoenicia.* The color of the corolla is not unlike that of *P. violacea* but the leaves, shape of flower and color of anthers are not true for the species. She says: "As it was found to propagate readily both by cuttings and by seeds, it soon spread over England, and became a general favourite." . . . "There are many hybrids and varieties of this species, as it is found to hybridize freely with the white Petunia."

From 1833 on recognized hybrids of these two species, of varying forms and often of great size and beauty, were described and illustrated by various authors in different countries.

Summary

1. A species is considered uncontaminated when for three consecutive generations, reckoning from the first seedlings grown, all the progeny have been true to type.

2. *Petunia parviflora* Juss. and *P. axillaris* (Lam.) B.S.P. (*P. nyctaginiflora* Juss.) are the species on which the genus *Petunia* was based.

3. *Petunia axillaris* and *P. violacea* Lindl. are the ancestors of the greater part, if not all, of the cultivated forms of *Petunia.*

4. The synonymy of the three species is discussed and the names as given above are accepted.

5. A detailed description of the three species, with full illustrations, is given.

6. The name *Petunia alba* Hort. has been given to a short-tubed, white-flowered strain of the cultivated *Petunia* which has bred true for twelve years.

7. The character of the pollen grains is believed to be of diagnostic value in *Petunia.*

8. Segregates from hybrids into which *P. violacea* has entered, even at a very remote period, may easily be mistaken for the species unless very care-

ully observed. This fact accounts for much of the confusion found in the literature relative to *Petunia*.

We beg to add that we will most gladly send seeds of *P. axillaris* (Lam.) B.S.P., of *P. parviflora* Juss. and of *P. alba* Hort. to anyone desiring them. Should we be fortunate enough to secure, in our cultures, freely fertile plants of *P. violacea* Lindl., the seeds of this species would be included.

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WELLESLEY, MASSACHUSETTS

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EXPLANATION OF PLATES

All drawings were made from living material grown in the open. The originals have been reduced slightly more than was intended when the magnifications were computed, so that the latter are about 5 percent too high. The figures, as shown, are natural size unless otherwise indicated.

PLATE XXXIII

Petunia axillaris (Lam.) B.S.P.

FIG. 1. A branch showing (*a*) young fruit, (*b*) wilting flower, (*c*) open flower, (*d*) flower bud, (*e*) young axillary branch, and leaves associated with the flowers.

FIG. 2. A basal leaf.

FIG. 3. Open corolla showing stamens and the characteristic markings of the throat.

FIG. 4 The relation of anthers to stigma. × 2.

FIG. 5. The pistil.

FIGS. 6 and 7. Front and top views of the stigma. × 8.

FIG. 8. Fruit just at the moment of dehiscence showing the greatly enlarged calyx-lobes.

FIG. 9. Mature fruit with the calyx-lobes dried and strongly reflexed.

FIG. 10. Seed. × 8.

PLATE XXXIV

FIGS. 11–22. *Petunia parviflora* Juss.

FIG. 11. A bit of young plant showing vegetative parts, flower, and fruit.

FIGS. 12 and 13. A flower from a young plant and one from an older plant showing differences in length of calyx-lobes; *a* indicates position of "fold" in limb.

FIG. 14. Face view of flower showing "lip" on anterior side caused by "fold" in limb at the line of its junction with the throat. × 2.

FIG. 15. Open corolla showing height of stamens. × 2.

FIG. 16. The relation of anthers to stigma. × 2.

FIG. 17. The pistil.

FIGS. 18 and 19. Back and top views of stigma. × 8.

FIG. 20. Upper portion of style with side view of stigma. × 8.

FIGS. 21 and 22. Mature fruit at time of dehiscence. Seed. × 8.

FIGS. 23–33. *Petunia violacea* Lindl.

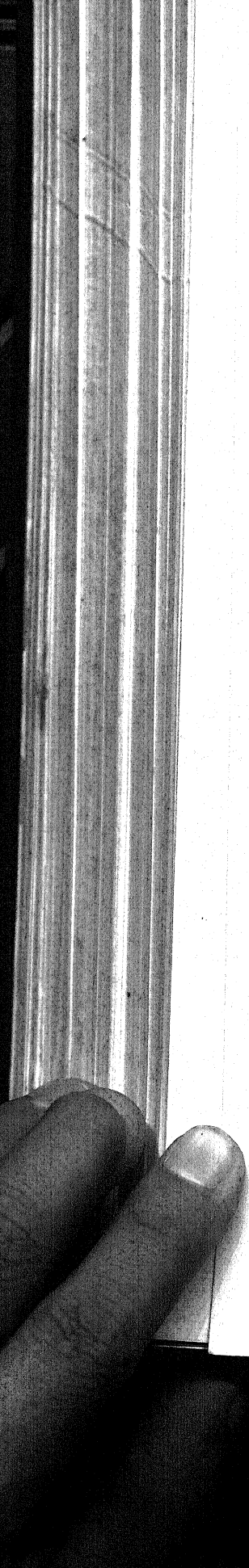
FIG. 23. Branch of plant; *a*, young fruit showing characteristic recurving of the peduncle. The flower was just beginning to wilt as indicated by the fold in the corolla.

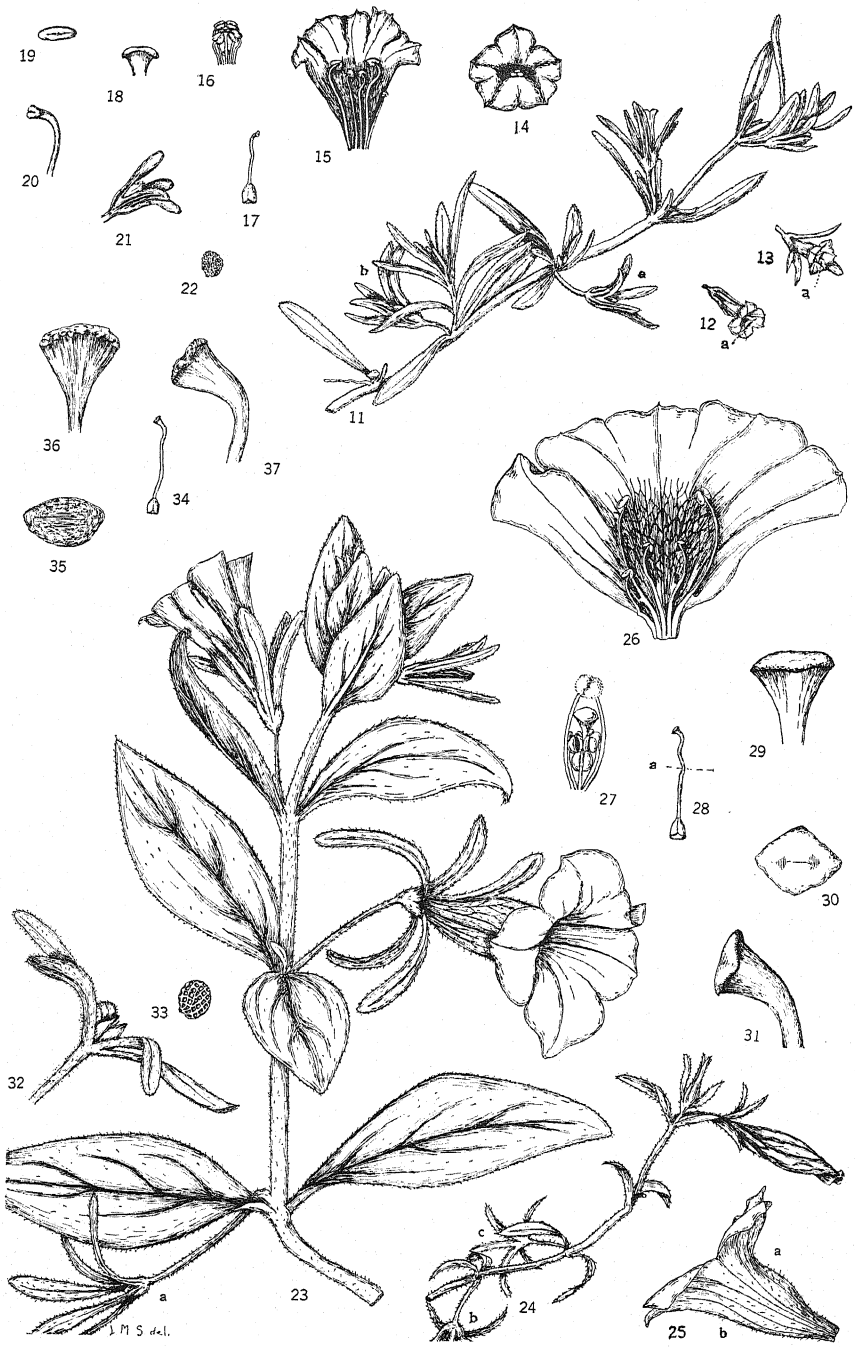
FIG. 24. A bit of the flowering portion of an old plant with vegetative parts so reduced as to suggest an inflorescence; *b* and *c*, reflexed fruits.

FIG. 25. Side view of corolla showing short tube and ventricose throat; *a* posterior, *b* anterior side of flower.

FIG. 26. Open corolla showing attachment of stamens, sagittate anthers and colored veining of the throat.

FERGUSON AND OTTLEY: PETUNIA





FERGUSON AND OTTLEY: PETUNIA

FIG. 27. The relation of anthers to stigma. × 2.

FIG. 28. The pistil. Dotted line *a* indicates the height at which the cut was made for FIG. 27.

FIGS. 29 and 30. Front and top view of stigma. × 8.

FIG. 31. Upper portion of style with side view of stigma. × 8.

FIGS. 32 and 33. Mature fruit at time of dehiscence. Seed. × 8.

FIGS. 34–37, Population 10. A chance seedling

FIG. 34. The pistil.

FIGS. 35 and 36. Top and front view of stigma. × 8.

FIG. 37. Upper portion of style with side view of stigma. × 8.

THE LOWER CALIFORNIA BUCKEYE, *AESCULUS PARRYI* A. GRAY

IRA L. WIGGINS

(Received for publication November 21, 1931)

At intervals it has been my privilege to observe this Lower California plant over the greater part of its known range. It first came to my attention in September, 1929, when it was collected in mature fruit thirty miles north of Ensenada and noted on the hills as far east as San Rafael Valley. In February and March of the following year it was studied over the length of its north and south range while the flowers were still in the bud. It was again seen in mature fruit in September, 1930, and in full flower in April and May, 1931. As an endemic of the northern part of Lower California its exact range was of more than usual interest, and certain peculiarities of the flower lead me to study it critically on each of four visits to its range. It is due largely to these field observations, made possible by the generosity of Mr. H. C. Dudley, of Duluth, Minnesota, and Mr. E. G. Dudley, of Exeter, California, that the following information concerning characters previously overlooked can be given and slight extensions of the range as reported by earlier collectors can be added. Their interest in the botanical exploration of Lower California and their generous aid is very greatly appreciated.

HISTORY OF THE SPECIES

Aesculus Parryi was discovered on the low hills near Todos Santos Bay, on the shores of which the town of Ensenada is situated, in April, 1882, by C. C. Parry, C. G. Pringle, and Marcus E. Jones. Specimens were sent to Asa Gray who described it and designated *A. californica* (Spach) Nutt. as its nearest relative. Gray's description reads as follows: [1]

"Aesculus Parryi, *Ae. Californicae* affinis, frutex humilis; foliis 3–5-foliolatis; foliolis obovatis obtusis subcoriaceis brevissime petiolulatis subtus cano-tomentosis; floribus brevi-pedicellatis; calyce campanulato ad medium usque aequaliter 5-fido petalisque extus tomentosis; filamentis validioribus minus exsertis.—Northern part of Lower California, April, 1882, *Parry*, *Jones*, and *Pringle*."

This description clearly separates the Lower Californian *Aesculus* from *A. californica*, but it does not take into account several important characters mentioned in the amplified description below.

The next mention of *A. Parryi* was made in 1889 by T. S. Brandegee [2] who reported its occurance at Rosario, a village 170 miles south of Todos

[1] Proc. Amer. Acad. 17: 200. 1882.

[2] Proc. Calif. Acad. II, 2: 143. 1889.

Santos Bay. He did not describe the plant, but merely noted its occurance at that point.

The following year C. S. Sargent [3] published a semipopular description of *A. Parryi* and accompanied his article with the only illustration published up to the present time. He was evidently satisfied with Gray's interpretation of the relationship of the species, but he did point out more clearly the characters which separated it from *A. californica*, and described the fruit which had been unknown to Gray. The following quotation is a part of Sargent's description:

"The chief differences are found in the character of the calyx, which in *Aesculus Californica* is deeply two-lobed, the lobes being very slightly divided, while in *Aesculus Parryi* the campanulate calyx has five nearly equal lobes. These characters are constant in all the species I have examined, and I fail to find any intermediate form which would serve to connect the plant of Lower California with the California Buckeye, from which it differs also in its shorter inflorescence, its shorter pedicels, smaller flowers and fruit, and in the dense pubescence which covers the under surface of the small obovate leaflets, which are sessile or nearly so. The fruit is unarmed, obovate, rather less than an inch long and broad, in the specimens I have seen, which are from one to three-seeded. The seed is a third of an inch in diameter."

It was not until 1897 that Robinson,[4] basing his suspicions upon the 5-cleft calyx, expressed a doubt as to the advisability of including *A. Parryi* in the section *Calothyrsus*, of which *A. californica* is the type. He made the following comment in a short note following his description of *A. californica:*

"Ae. parryi, Gray, is a related species of N. Lower California, but on account of its 5-fid calyx scarcely to be referred to this section."

In 1916 Goldman [5] reported *A. Parryi* from San Rafael Valley east of Ensenada and mentioned its zonal distribution in connection with its southern limit. He gave no description of the flowers or fruit but gave the height of the shrub as 1.2 to 2.5 meters.

The last published account of this shrub was given in 1923 by Standley,[6] who included measurements of leaves, inflorescence, and calyx in his brief description.

Relationship and Description

After carefully studying this species in the field and herbarium I am convinced that *A. Parryi* exhibits too many characters not possessed by other species of the established sections of the genus to warrant retaining it in any of them. The description given below indicates the degree to which *A. Parryi* has diverged from the other stocks during the evolution of the

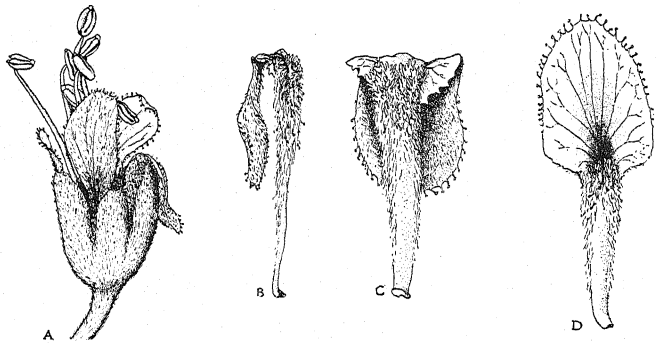
[3] Gard. & For. 3: 356. *f.* 47. 1890.
[4] Syn. Fl. N. Amer. 1: *pt.* 1, 448. 1897.
[5] Contr. U. S. Nat. Herb. 16: 346. 1916.
[6] Contr. U. S. Nat. Herb. 23: 691. 1923.

genus. In order that this peculiar endemic species of Lower California may be coördinately ranked with equally distinct species or groups of species in the classification of the genus, the following new section is proposed.

Parryanae, new section. Calyx campanulate, 5-cleft, lobes subequal, tomentose and stipitate-glandular; petals 4–5, stipitate-glandular on the margins, tomentulose on the back, bearing laciniate appendages at the summits of the claws; fruit tuberculate-roughened, tomentose.

Represented only by the following species:

Aesculus Parryi A. Gray, Proc. Amer. Acad. 17: 200. 1882. Shrub or small tree 1–6 m. high, trunk up to 2 dm. in diam.; bark smooth, gray to nearly white on old trunks; winter buds not resinous; leaves on petioles 5–10



TEXT FIG. 1. *A*, sterile flower with reflexed upper petals and erect lower petals. *B–C*, lateral and face view of upper petal with the appendages and dense pubescence at summit of the claws. *D*, lower petal; the shaded portion of the blade indicates the approximate extent of the maroon spot at the base of the blade. *A*, × 2; *B–D*, × 4.

cm. long, 3–7-foliolate, or occasionally 1-foliolate by the reduction of the lateral leaflets and then resembling a long-petiolate simple leaf; leaflets 3–12 cm. long, obovate, oblong-obovate or oblong-elliptic, obtuse or acute, sessile or short-stalked, entire to moderately repand, bright green above, white-tomentose beneath; panicle narrow, 5–20 cm. long, fertile flowers scattered full length of the inflorescence; calyx campanulate, 7–8 mm. long, 5-cleft to the middle, the lobes obtuse, the upper slightly longer than the two lower, the lateral lobes $\frac{1}{2}$ to $\frac{2}{3}$ as long as the upper, finely and densely tomentulose, stipitate yellowish glands intermingled with the wool, margins stipitate-glandular; petals 4–5, narrowly obovate, acute or obtuse, creamy white with a bright maroon spot at the base of the blade, glandular-stipitate but not hairy on the margins, tomentulose on the backs, the claws equaling or slightly shorter than the calyx lobes, bearing 3–8 laciniate flap-like appendages at their summits; 2 upper petals 12–14 mm. long, strongly reflexed, the lower 8–11 mm. long, erect or slightly spreading but not reflexed; disk developed more strongly on the upper side of the flower than on the lower, and more pronounced in sterile than in fertile flowers; stamens 6–8, exserted;

filaments 10–16 mm. long, the tips curved upward; anthers bright orange; pistils of sterile flowers equaling the calyx lobes, those of fertile flowers equaling or slightly surpassing the stamens; functional ovaries densely stipitate-glandular and tomentose; fruit borne full length of the inflorescence, unarmed, spherical to obovate, 15–25 mm. long, tuberculate, tomentose and slightly glandular, light brown, 1–3-seeded, occasionally both ovules in a cell maturing; seeds 12–16 mm. in diameter, dark chestnut-brown to nearly black; hilum white or light buff, 3–4 mm. in diameter.

The non-resinous winter buds, 5-cleft calyx and glandular-ciliate, appendaged petals clearly separate *A. Parryi* from the section *Calothyrsus*, in which Rehder [7] places *A. californica* and *A. wilsonii*. The bud character, the 5-cleft calyx and the red-spotted, glandular-margined petals indicate a closer relationship with the *Eupaviae* series of the section *Pavia*, but the appendages on the petals, the shorter claws, the campanulate calyx and the smaller, woolly flowers remove it from that section also. It seems to me that its ancestry should be looked for in the forms giving rise to the *Eupaviae* group which has leaflets which are tomentose on the lower surface and glandular-ciliate petals, rather than with any other section of the genus.

A. discolor, a member of the *Eupaviae* group which comes westward from Georgia into Texas possibly represents another divergent branch from the same ancestral stock, but it has produced larger flowers and fruit which are nearly or quite glabrous and the petal appendages are not found in *A. discolor*.

Geographical and Zonal Distribution

The most northerly specimens of *A. Parryi* so far observed are growing on the coastal slope near the ocean in the vicinity of La Salina Rancho thirty miles north of Ensenada. Scattered specimens occur on the hillsides between these two places, but they are not abundant until one approaches Ensenada. Here the hills support numerous thickets of *A. Parryi*, the bright tomentum on the lower surface of the leaflets flashing silver whenever a breeze stirs the foliage. From Ensenada it extends eastward to the San Rafael Valley in the vicinity of Ojos Negros where a few dormant specimens were seen in September, 1929. Goldman reported it from the same vicinity in 1916, having noted its presence there in 1905.

It also occurs at a considerable distance from the coast in the vicinity of San Telmo and San Quintin, where it is found in the foothills of the San Pedro Martir, a distance of thirty miles or more from the ocean. It reaches its finest development, however, near the sea, becoming a tree 6 meters high with a trunk 2 dm. in diameter on the mesas just north of Rosario.

The known southern limit of *A. Parryi* is a point about half way between Rosario and the ruins of the old mission at San Fernando. In this region it is confined to the north slopes of the hills and along the arroyos and does not appear on the flat tops of the mesas as it does about Rosario nor on the flats along the arroyos as it does at Santo Domingo and Colnett.

[7] Manual of cultivated trees and shrubs 579, 580. 1927.

A. Parryi is confined to the lower part of the Upper Sonoran Zone, in some places seeming to be a border-line plant between the two zones. Between Mission San Vicente and San Antonio del Mar (Johnson's Ranch) it grows over a great part of a broad, gently sloping inland valley where it associates with *Opuntia prolifera, Agave spp., Ephedra californica,* and *Fraxinus dipetala trifoliata* in islands surrounded by grasses and herbaceous annuals. It does not ascend into the *Quercus dumosa-Arctostaphylos bicolor* complex to any appreciable degree, associating with these and their associated shrubs only where stragglers intermingle along the zone of contact. The shrubs with which *A. Parryi* most frequently associates over the greater part of its range include the following: *Euphorbia misera, Rhus laurina, Rhus integrifolia, Cneoridium dumosum, Fraxinus dipetala trifoliata, Prunus ilicifolia, Acalypha californica, Toxicodendron diversifolium, Rosa minutifolia,* and *Simmondsia californica.*

Specimens Examined

The following specimens of *A. Parryi* were examined in the herbarium during the course of the study and compared with specimens of other species of the genus. In addition to the notes made in the field, material was colected in alcohol and brought back for more careful dissection and study in the laboratory. Material from the Herbarium of the University of California was available, and specimens deposited in that herbarium and not in the Dudley Herbarium at Stanford University are designated by the abbreviation (U. C.). Specimens not so designated are in the Dudley Herbarium.

Specimens examined: El Rosario, *Brandegee,* May 20, 1889; San Quintin Bay, *Mason 2051;* Aliso, *Brandegee,* May 30, 1893 (U. C.); no locality, but probably from the vicinity of Ensenada, *Parry,* January, 1883 (U. C.); Ensenada, *Johnston 3013* (U. C.); near La Salina Rancho, foot of Guatay Grade, about 4 miles south of Rio Gudalupe, *Wiggins & Gillespie 3919;* flats along Rio Santo Domingo, near Hamilton Ranch, 14 mi. N. of San Quintin, *Wiggins 4491;* Las Animas Canyon, 20 mi. S. of Ensenada, *Wiggins & McMurphy 5170.*

Dudley Herbarium,
Stanford University,
California

CHROMOSOME MORPHOLOGY AND MEIOTIC BEHAVIOR IN TYPICAL AND VARIANT FORMS OF *KNIPHOFIA ALOIDES*

J. M. WEBBER

(Received for publication November 27, 1931)

INTRODUCTION

Under cultivation species of *Kniphofia* are known to hybridize freely and produce variable seedlings. Since these seedlings are propagated and distributed by vegetative means, the taxonomic relations are difficult to trace, and hence there is considerable confusion in the literature dealing with the genus. Probably the most variable species of the genus is *K. aloides*. According to Baker (2) there are perhaps ten to twelve botanical varieties and at least twice as many garden forms of this species.

Kniphofia aloides has been reported by de Vilmorin and Simonet (17); Belling (3), and Fernandes (10) to have six pairs of chromosomes. Fernandes reports the chromosome morphology to be 2Ll, 4LK and 6LK (E. Heitz 12). Although such a formula is rather vague, it indicates that morphological distinctions are present in the chromosome complement. It has frequently been demonstrated that such distinctions may be characteristic of a genus, species, or even an individual form. Consequently it appeared that cytological features would aid in establishing criteria of systematic value. The present study is an effort to obtain evidence by cytological means as to the origin and relationship of several forms of *Kniphofia aloides*.

MATERIAL AND METHODS

The material for this study was largely collected from residential gardens in Berkeley and from the University of California Botanical Gardens. Some material was also obtained from seed acquired from the Muséum d'Histoire Naturelle, Paris. Unfortunately, the rootstocks were set out at different seasons and the plants grown under diverse environmental conditions. These circumstances lead the plants to develop and flower at different times. Hence it was impossible to make a careful comparison of the external morphological characters of the different plants. Of the seven plants studied KB–3 and KB–4 were noted as phenotypic variants. KB–3 was dwarfed in habit and produced shorter spikes with fewer flowers. KB–4 was more vigorous than average in habit and produced larger spikes and flowers. Both of these plants were from residential gardens. The remaining five plants were typical of the species.

Root tips and anthers were fixed in chrom-acetic-formalin and stained in Haidenhain's iron-haematoxylin. Aceto-carmine and Brazilin smears (18)

were also employed in the preparation of the microspore-mother-cells and the male gametophytes. The results obtained from the slides prepared by the various methods are in perfect agreement.

Kniphofia aloides is unusually well suited for studies of chromosome morphology and structure, and the processes of microsporogenesis. Preparations showing the formation of microspores and male gametophytes are easily obtained. Stages showing the formation of generative and tube nuclei afford excellent material for the study of chromosome morphology and structure. Observations on the latter stage agree in nearly every detail with those of the mitotic divisions in the root tips.

This species has the added advantage of having flowers borne in long spikes. The buds develop successively at the apex of the indeterminate spike so that the first matured flowers occur at the base. Hence the material can be collected in systematic order. The average number of buds from ten spikes in definite stages of division are given in table 1. This table indicates the comparative length of the periods involved during the various stages, and shows that it would be a rather simple matter to determine the approximate times to collect any desired stage.

TABLE 1. *Average Number of Buds from Ten Spikes in Definite Stages of Division. (Order from Apex Downward)*

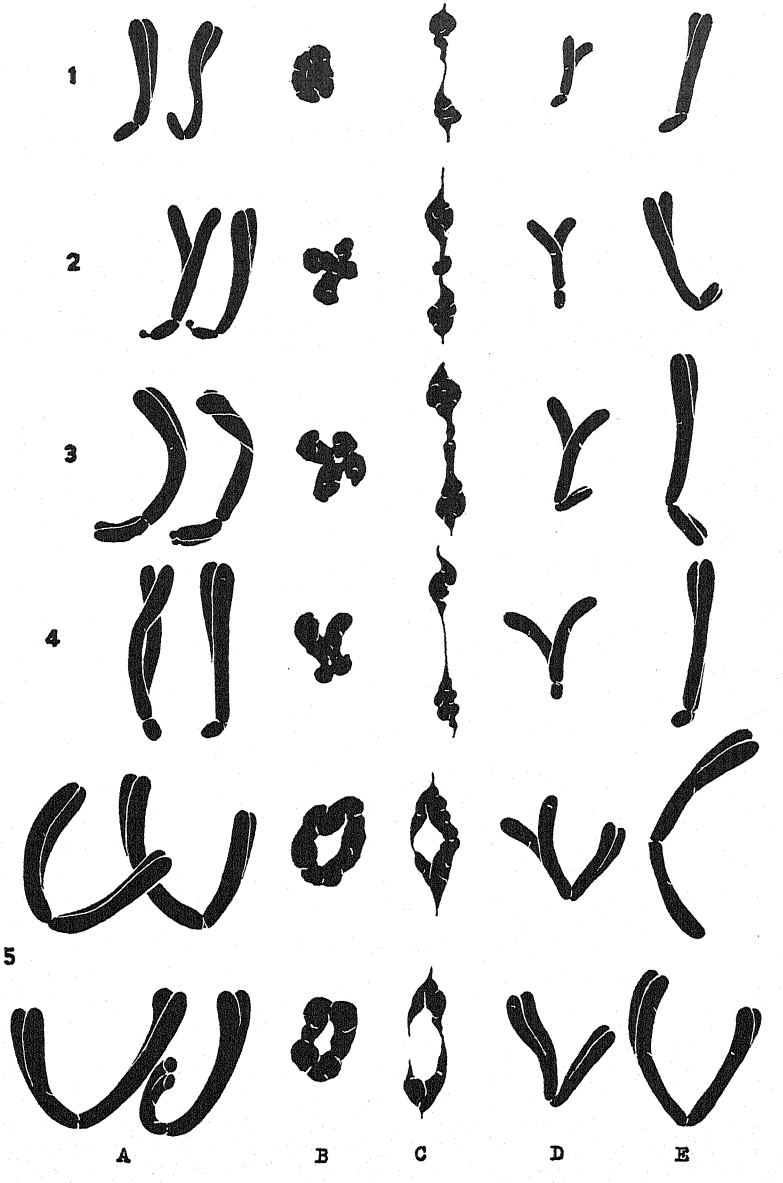
Archesporial and thread stages	About 30 buds
Diakinesis	3 buds
First Metaphase	1.5 buds
First Anaphase (Lengthening period)	2 buds
First Anaphase (Contraction period)	0.5 buds
Interkinesis	2.5 buds
Second Metaphase	1 bud
Second Anaphase	2 buds
Telophase	2 buds
Tetrads	12 buds
Young Microspores	2.5 buds
Male gametophyte (formation of tube and generative nuclei)	2.5 buds
Matured Pollen	Many

CYTOLOGY OF TYPICAL FORMS

Somatic Chromosomes

In the most favorable somatic metaphase plates from root tips, Plate XXXV, figure 9 and text figure 2, it is possible to identify five types of chromosomes. Type 1, text figure 1, consists of a small pair with sub-terminal constrictions. The short segments of this pair average about 2.09 μ in length and the long segments about 3.95 μ. Type 2 likewise consists of a small pair of chromosomes with sub-terminal constrictions. The larger segments of this pair, however, average 1.66 μ longer than the larger segments of the preceding pair. This pair also has well defined proximal satellites. The remaining chromosomes of the complement are considerably larger.

The chromosomes of type 3 consist of a sub-terminally constricted pair, the segments of which average about 2.78 μ and 6.95 μ in length. Although the members of this pair have not been observed to form well defined satellites, they frequently form small knobs or pseudo-satellites at the



TEXT FIG. 1. 1–5, the five different chromosome types of *Kniphofia aloides*. *A*, root tip metaphase chromosomes. *B*, P.M.C. diakinetic pairs. *C*, characteristic anaphase separation in P.M.C. *D*, P.M.C. second metaphase chromosomes. *E*, chromosomes of male gametophyte. × 2800.

proximal ends. The longitudinal halves of each long segment of this pair often tend to twist about each other in rope fashion, thus giving the appearance of a second constriction. Such constrictions may readily be mistaken for spindle fiber constrictions, especially if the chromosomes bend at the point of the twist or if the material is over-stained.

Type 4 is likewise composed of a sub-terminally constricted pair. In this pair the segments average 1.80 μ and 6.85 μ in length. Type 5 consists of two pairs of chromosomes constricted near the middle. Since the form and size of these four chromosomes is practically the same, they cannot with certainty be separated into pairs. The several measurements of length of the shorter segments varied only from 4.99 to 5.10 μ and the larger segments from 6.95 to 7.12 μ.

From the preceding type descriptions, it is apparent that the chromosome complement may be divided into a group of "J" shaped and a group of "V" shaped chromosomes (text fig. 1). To the former group belong types 1, 2, 3 and 4 with sub-terminal constrictions and in the latter group, type 5 with sub-median constrictions. The complement may also be divided into small chromosomes, type 1; medium, type 2; and large, types 3, 4 and 5. These groupings are of special interest, since, as will be described below, they exhibit distinct behavior during meiosis.

The point of constriction apparently determines the chromosome orientation in the metaphase plate. The large "V" shaped chromosomes are arranged at the periphery and lie quite flat, while the "J" shaped chromosomes, especially those which approach a rod shape, are arranged in the center of the plate and tend to turn away from the horizontal position (text fig. 2). Such a situation may account for the fact that the chromosomes often have the appearance of being oriented in pairs.

Meiotic Chromosomes

Since a study of the thread stages has not yet been completed, only the behavior from diakinesis onward will be discussed.

During diakinesis there are formed two large rings, two large "X"s, one medium sized "X" and one smaller droplet of double appearance (Pl. XXXV, fig. 1, and text fig. 1). Although these configurations vary somewhat in shape and size, the variations depend upon the state of contraction. Thus, during early diakinesis the rings are large enough to loop and often appear as two small joined rings. Such loops, however, are straightened by contraction and are never found at late diakinesis. Hence, complete configuration stages are most frequent and favorable during late diakinesis.

Although the size relations of the diakinetic pairs correspond with those previously described for the somatic pairs, a relationship between diakinetic forms and somatic forms is much less consistent. Nevertheless, some analogy does exist. In the somatic garniture there are two pairs of "V"s and four pairs of "J"s. It seems probable that the two diakinetic rings represent

the somatic "V"s while the three diakinetic "X"s and double droplet represent the "J"s. The existence of such a form relationship is further supported by the fact that in late second contraction there are four meiotic "X"s instead of three, as found in diakinesis. It is also strongly supported by the behavior found in abnormal forms described below.

During the first metaphase, the bivalents, with the exception of one pair, are globular in shape and compose a very regular plate. The exceptional pair has a tendency to retain its diakinetic form and lie slightly apart from the other five pairs (Pl. XXXV, fig. 2). Although at this stage, one pair is considerably smaller than the others, size relations are not as distinct as in the case of the somatic and diakinetic pairs. The small bivalent probably represents the small somatic pair, type 1.

In the anaphase the bivalent partners do not disjoin in the ordinary way, nor pass to the poles in orderly rows of individual members. The partners go through a process of lengthening until their points of spindle fiber attachment have reached the polar regions (Pl. XXXV, fig. 3). During this process the partners remain attached to each other at their equatorial ends and become nearly the same length as the somatic chromosomes. They also become very knotty and often resemble a corkscrew. The attachment between the partners breaks at late anaphase and the partners contract very rapidly (see table 1) to the opposite poles (Pl. XXXV, fig. 4). This contraction results in the chromosomes entering interkinesis in the form of globules.

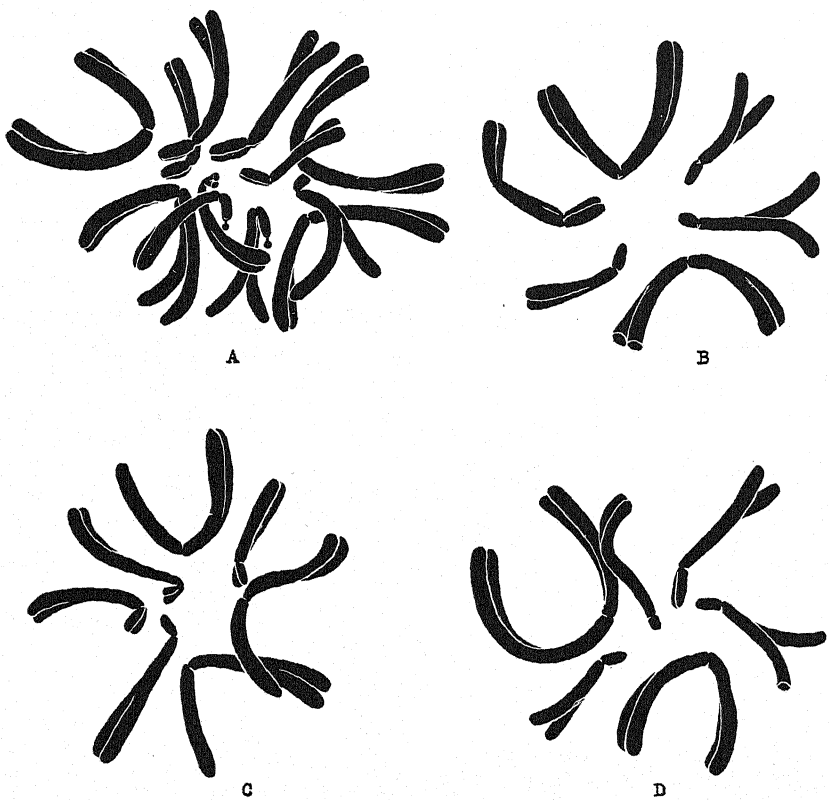
The above anaphase process does not take place simultaneously in all the chromosomes. Only four bivalents start disjunction in early anaphase. The partners of these four bivalents become more or less rod shaped as the separation proceeds. In this group there is one small pair, one medium sized pair and two large pairs (text fig. 1). During separation the medium sized pair develops a large knot at the equatorial region. This knot persists until the pair is finally separated in late anaphase. These four pairs undoubtedly represent the somatic "J" shaped groups of chromosomes. Probably the small pair represents the somatic type 1; the medium sized pair, the somatic type 2 with satellites; and the two large pairs, the somatic types 3 and 4. When the free ends of the above four pairs are about half way between the equatorial zone and the polar regions, the remaining two pairs begin to disjoin. The partners of these two bivalents are at first very massive and "V" shaped. Both ends of the synaptic "V"s are attached to each other and hence the pairs often appear diamond shaped (text fig. 1). During their lengthening period the V shape becomes more pronounced and the chromosomes may become detached at one end. These two large pairs probably represent the four large somatic V-shaped chromosomes, type 5.

Although a detailed study of interkinesis has not been made, it has been noted that the chromosomes pass through a series of changes. They lose their identity, the chromatin becomes rather finely dispersed, and finally a

spireme is formed. From this spireme the chromosomes emerge in their somatic form.

During the second metaphase the chromosomes are usually twisted in such a fashion as to make study difficult (Pl. XXXV, fig. 5). In an occasional plate, however, they are exceptionally well spaced and the five described somatic types are very clear. Although within the second metaphase group the size relations of the types are the same as within the somatic complement (text fig. 1), the whole group is considerably smaller. Such a condition may account for the more definite constrictions in the second metaphase chromosomes. In no case has type 2 been observed to form a satellite during second metaphase.

In the remaining stages of microsporogenesis nothing of particular interest in connection with the present report was observed. However, it might be stated here that cytokinesis is accomplished by furrowing at the end of second division (Pl. XXXV, figs. 6 and 7).



TEXT FIG. 2. *A* and *B*, typical *Kniphofia aloides*. Metaphase plates of root tip and male gametophyte. *C*, variant KB-4. Metaphase plate of male gametophyte. *D*, variant KB-3. Metaphase plate of male gametophyte. × 2600.

Gametophytic Chromosomes

During the formation of the tube and generative nuclei, the morphological characteristics of the six haploid chromosomes are exceedingly clear (Pl. XXXV, fig. 8, and text fig. 2). In the metaphase the chromosomes usually lie quite flat and the five described somatic types are easily distinguishable. The average measurements of the types at this stage are practically identical with those of the somatic averages. Although type 2 occasionally tends to form a satellite, it frequently, as in the case of second metaphase, fails to form one. The types are likewise easily distinguishable during the anaphase.

A limited study of chromosome morphology and behavior during the development of the female spore and gametophyte has revealed that it is practically identical with that described in the case of the male.

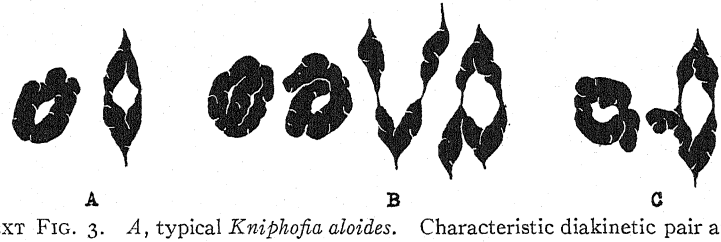
Cytology of Variant Forms

Since the five chromosome types of *Kniphofia aloides* are easily distinguishable during meiosis and the first division within the male gametophyte, the somatic complement of the variant forms has not been studied.

Variant KB-4

This form is characterized by having enlarged external morphological characters. Upon cytological examination it was found to be a trisomic form.

During diakinesis and first metaphase in the microspore-mother-cells of KB-4 there are generally formed five bivalents and one trivalent. Occasionally, however, one of the three homologs of the trivalent fails to join or remain attached to the others. In this case, the univalent is well separated from the bivalent, even to the extent of being situated on the opposite side of the nucleus or plate.



Text Fig. 3. *A*, typical *Kniphofia aloides*. Characteristic diakinetic pair and mode of separation of the fifth type chromosomes. *B*, variant KB-4. Characteristic trivalents and modes of separation. *C*, variant KB-3. Characteristic pair involving the modified fifth chromosome and the mode of separation. × 2800.

When six pairs are present, the typical diakinetic configurations are clearly represented. The additional univalent, which occurs in the latter case, is more or less rod shape and about one-half the size of one of the large rings. When the trivalent is formed it replaces one of the two large rings and assumes one of two forms (text fig. 3). The most common of these

forms is a ring with the extra homolog attached by one end and bending either toward or away from the ring. There is little variation in the second trivalent form, the ends of the extra homolog being attached to opposite side of the ring.

During first metaphase the trivalent is easily distinguished from the bivalents by its large size and its more or less three lobed character. Likewise, the univalent is smaller than the bivalents. As in the typical forms, described above, only two of the metaphase bivalents are distinguishable from the others. These two are the rather small pair and the pair which retains its diakinetic form. Hence during this stage it is impossible to identify the pair with which the extra homolog associates. However, since the two bivalents which can be identified occur in plates along with the trivalent, it is certain that the extra chromosome is not associated with either of these pairs.

During the first anaphase the bivalent partners behave like those in the typical form. The trivalent partners, as in the case of diakinesis, are either joined at both ends or the end of one mate is free (text fig. 3). These partners become V-shaped during separation and are distributed along with the products of bivalent disjunction. On the other hand, when the univalent is formed it exhibits no definite shape and is usually distributed at random. It frequently lags, is often left in the plasma and occasionally divides. Very rarely the univalent forms a thread-like connection between the two late anaphase groups. This connection apparently results in the suspension of the division and finally in the production of somatic gametes.

The second metaphase counts confirm the anaphase findings, that is, occasionally the extra chromosome has been eliminated, rarely somatic metaphases have been formed and very rarely the univalent has divided. In all favorable plates which contain only six chromosomes the five haploid somatic types are easily distinguishable. In plates which contain seven chromosomes the five types are likewise distinguishable but there are three chromosomes of type 5 instead of two. In one somatic metaphase plate it was possible to classify all thirteen chromosomes into their respective types. There were clearly five chromosomes of type 5.

That univalent division at first anaphase was actually a fragmentation is shown by the extreme variation in size of resulting parts. The length in microns of several fragments is recorded in table 2.

TABLE 2. *Length of Univalent Parts at Second Metaphase* *

Single plates	1.30	2.10	3.84	5.00	6.02	6.01	7.00
Both plates	*3.75* 5.24	*2.06* 6.95	*2.25* 6.60	*1.02* 7.79	*4.45* 4.65	Average	
Total length both plates	8.99	9.01	8.85	8.81	9.10	8.95	

* Only single divisions into two parts occurred.

The average length of both fragments occurring in single microspore-mother-cells was 8.95 μ. This average agrees fairly well with 8.99 μ, the average length of the fifth type chromosome at this stage. In only the first case in table 2 was the break approximately at the point of constriction. Only rarely do the fragments exhibit constrictions.

A detailed study of second anaphase has not been made. However, the division is fairly regular except for a very slight amount of lagging. This lagging in all cases appears to be concerned with the fragments. It is likely that in a few of the fragments the chromatin has been reorganized by fragmentation, so that a normal homotypic division is no longer possible. Such a change may involve only the spindle fiber constriction.

Chromosome counts during the first division within the male gametophyte confirm the above meiotic behavior. The four expected types, n, n + a fragment, $n + 1$ (text fig. 2), and $2n + 1$, of gametophytes were observed. No gametophytes which might have resulted from the extra chromosome being eliminated in the plasma were observed. The chromosome morphology during this stage agrees with that of second metaphase, the extra chromosome belonging to the fifth somatic type.

The gametophytes which contain extra chromosomes are apparently as vigorous as those which contain the normal haploid number. The gametophytes which contain the somatic chromosome complement may be readily distinguished by their large size.

Variant KB-3

External morphological characters of this form are slightly dwarf. With the exception of one chromosome KB–3 was found to have the typical somatic complement and exhibit normal meiotic behavior. The exceptional chromosome belongs to the fifth type of somatic chromosomes and is considerably longer than its homolog. Since the pair involving this long chromosome does not exhibit irregularities during meiosis, only the features bearing upon its possible origin will be described. These features are the degree or closeness of diakinetic and first metaphase conjunction, the diakinetic configuration, and the length of the modified chromosome.

During diakinesis the modified chromosome is united to its partner at one end and in the region where its other end would normally be located (text fig. 3). The remaining part or additional length of this long homolog remains attached in the form of a small bent rod. The whole configuration is more or less "Q" shaped. The ring shaped part of this figure is of the same general size and shape as that in the typical form. The affinity between the homologs is also equal to that in the typical case. In shape and behavior it greatly resembles the first trivalent discussed in the case of KB–4.

During the first metaphase the modified pair is fairly easily distinguished from the normal bivalents by its large size. However, it differs from the KB–4 trivalent in that it does not exhibit the three lobed appearance. Hence, the affinity between the homologs is probably greater than that

where extra homologs are present and is probably equal to that in the typical form.

At second metaphase the segments of the modified chromosome average 5.30 μ and 7.55 μ. The average of the shorter segment agrees fairly well with the 5.24 μ average of the longer segment in the typical form. The long segment is about twice, 3.75 μ, that of the shorter segment in the typical form. These relations agree with those found in the male gametophyte. During the first division in the latter case the modified chromosome segments average 7.01 μ and 10.40 μ,(text fig. 3). In the typical form the measurements are respectively 6.99 μ and 5.00 μ.

Discussion

In the preceding cytological description it is shown that eight of the twelve chromosomes of *Kniphofia aloides* are readily separable into pairs. The remaining four chromosomes, constituting type 5, exhibit identical or nearly identical morphological features and meiotic behavior. Since they never form multivalents during meiosis, they are clearly members of non-homologous pairs.

Variant form KB–4 is shown to have in addition to the typical complement one extra, or five chromosomes of the fifth type. These chromosomes usually form a bivalent and a trivalent during meiosis. Variant form KB–3 involves the same group of chromosomes. It has the typical complement with the exception of one extra large chromosome of the fifth type. Measurements of this large chromosome show that one of its segments is approximately twice as long as in the typical form. During meiosis it always pairs with one of the other chromosomes of the group. Such cytological affinity and morphological similarity indicate that this large chromosome probably has a duplicated section.

Similar behavior of supernumerary homologs has frequently been demonstrated by Belling (4), Belling and Blakeslee (6), and others. Duplication or the addition of a segment to a homolog (Bridges, 9) has not sufficient cytological basis. It is, however, clear that duplication is very similar to chromosome linkage (cf. *Datura*, *Oenothera*, *Aucuba*, etc.)

In view of the known behavior of supernumerary and modified chromosomes (cf. Goodspeed and Avery, 11), the similarity of morphological features and meiotic behavior of the extra and large chromosomes of the above variant forms to that of the fifth type chromosomes in the typical form, indicates unquestionable homology. It is therefore highly probable that the variant forms KB–4 and KB–3 were derived either from the typical species or from closely related species or forms.

Of the numerous methods by which these chromosome variants may have arisen it seems probable that polyploidy or nondisjunction was originally involved. In the preceding cytological description it is shown that KB–4 frequently forms gametes having the somatic chromosome complement.

The probable viability of these gametes is indicated by the apparently tetraploid nature of several of the larger variant forms of *Kniphofia aloides*.[1] It is well known that trisomic individuals are readily obtained from polyploid plants and polyploid hybrids (Belling and Blakeslee, 5; Van Overeen, 16; Lesley, 13; Webber, 19). Likewise, the production of trisomic individuals following nondisjunction has frequently been reported (Blakeslee and Belling, 8; Ruttle, 15; Avery, 1).

Fragmentation of unpaired chromosomes similar to that described above in the case of *Kniphofia* variant KB–4 has previously been reported in tomato and *Nicotiana* (J. W. and M. M. Lesley, 14; Webber, 19). Likewise, in the case of certain plants, diminutive chromosomes have been observed in individuals derived from parents known to form fragments (cf. preceding authors). It is conceivable that such fragments may become permanently attached to their homologs (Goodspeed and Avery, 11).

Further investigations of other forms of *Kniphofia* are being carried on and will be reported at a later date. The present study indicates that a relatively high percentage of these forms will prove to be chromosome variants. This high percentage of chromosome variants is readily explainable on the basis of polyploidy, hybridization and vegetative propagation.

Summary

1. The twelve somatic chromosomes of *Kniphofia aloides* can be separated into five types on the basis of size distinctions, position of constrictions, and occurrence of satellites.

2. The five types may readily be distinguished during meiotic divisions by form and characteristic modes of pairing. They are also distinguishable during the first division within the male gametophyte.

3. Of the seven plants examined KB–3 and KB–4 are chromosomal variants. The chromosomes involved in these variants are apparently homologous to chromosomes in the typical form.

4. KB–3 has one extra large chromosome, which is probably a normal homolog with a duplicated section. KB–4 is a trisomic plant.

5. These two variants probably originated from the typical form or from a closely related form by methods involving polyploidy or non-disjunction and fragmentation.

6. Due to hybridization and vegetative propagation there is likely to be a relatively high percentage of chromosome variants among the various forms of *Kniphofia aloides*.

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[1] Preliminary cytological examination of several forms of *K. aloides* indicates that they are tetraploid.

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EXPLANATION OF PLATE XXXV

Unretouched photomicrographs of Typical *Kniphofia aloides* (× approximately 650). FIG. 1. P.M.C. Diakinetic configuration. FIG. 2. P.M.C. first metaphase. FIG. 3. P.M.C. first anaphase, lengthening period. FIG. 4. P.M.C. first anaphase, contraction period. FIG. 5. P.M.C. second metaphase. FIG. 6. P.M.C. second anaphase. FIG. 7. Tetrads. FIG. 8. First metaphase male gametophyte. FIG. 9. Root tip metaphase plate.

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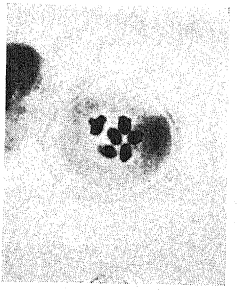

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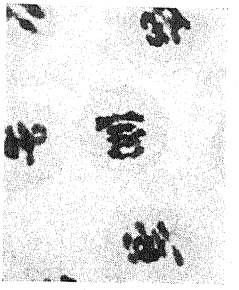

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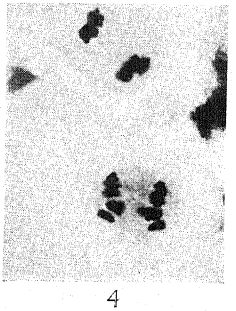

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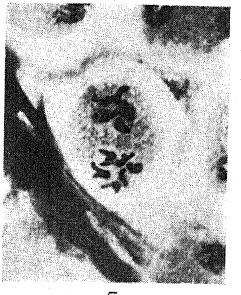

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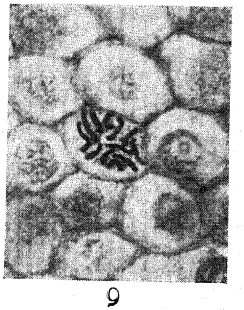

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WEBBER: CHROMOSOMES OF KNIPHOFIA

THE DEVELOPMENT OF THE PELTATE HAIRS OF *SHEPHERDIA CANADENSIS* [1]

D. C. COOPER

(Received for publication December 3, 1931)

It is well known that the characteristic silvery or reddish-brown appearance of the young twigs, buds, and leaves of the members of the Elaeagnaceae is due to a thick coating of peltate and stellate hairs. An examination of three members of this family, *Shepherdia canadensis* (L.) Nutt., *S. argentea* Nutt., and *Elaeagnus angustifolia* L. has shown the peltate hairs to be almost constant in size and shape within each species and very similar for the species examined.

The peltate hair may be described as consisting of a central stalk bearing a flattened or conical shield. The shield is made up of a considerable number of ray cells which usually have somewhat thickened walls and empty cell cavities. The distal end of each of these ray cells is free and tapers to a point.

It has been suggested by earlier investigators that the presence or absence of peltate hairs, and, if present, the form, size, and color of such hairs could be used in distinguishing between genera and species of plants. Schacht (1852, 1856) found that the scales (*Schuppen*) of the Elaeagnaceae were very similar to those of the Bromeliaceae. Prillieux (1856) separated some 23 members of the Oleaceae on the basis of the type of "star" hairs (*poils en étoile*) characterizing each species. He found that the hairs differ in size, shape, and color, and in the number of cells in the shield. He considered the peltate hair to be actually a stellate hair, in which the radiating cells of the shield are held together by a cuticle. De Candolle (1856) used the presence and form of the peltate hairs (*radiota pilosae*) as taxonomic characters in describing the members of the Elaeagnaceae. Vesque (1882) made use of various types of peltate hairs (*poils en écusson*), stellate hairs (*poils en étoile*) and bunched hairs (*poils en pinceau*) as taxonomic characters in the Capparidaceae. Haberlandt (1884) described the scale hair (*Schuppenhaar*) as characteristic of members of the Elaeagnaceae.

According to Bachmann (1886), Radlkofer (1884) observed a similarity in the structure of the peltate hairs of the members of four families of plants. Bachmann (1886) studied the peltate hairs (*Schildhaaren*) and the stellate hairs (*Sternhaaren*) of some 34 families of Angiosperms. On the basis of the characters of the peltate hairs, he formed a key to the genera of the

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin No. 128. Published with the approval of the director of the station.

families studied. He classified 20 members of the Elaeagnaceae as having shield hairs, only a portion of whose radiating cells reach from the center to the margin. Gilg (1894), Trelease (1925, 1926), Rehder (1927), and others have made use of the varying types of peltate hairs in describing various species of Elaeagnaceae. Rehder (1927) noted that the scales, which are divided to the base in *Shepherdia canadensis* and are merely dentate in *S. argentea*, are divided fully half way to the middle in the hybrid *S. argentea* × *canadensis* = *S. gottingensis*.

Sakisaka and Suehiro (1929) report two distinct types of "star" hairs in five Japanese species of *Elaeagnus*. In each species they find large hairs with a maximum diameter of 0.5 mm. and smaller hairs with a maximum diameter of 0.2 mm. covering leaves, twigs, sepals, and fruits. Hairs of the larger type in these species are considered to be sufficiently different in form to be used as taxonomic characters, whereas the smaller ones are useless for this purpose. The illustrations of the "star" hairs indicate that the authors have reference to the peltate form of hair.

It is perhaps possible that the peltate hairs of the species under consideration might likewise be used as taxonomic characters. It should be pointed out, however, that the peltate hair of *Elaeagnus angustifolia* (Pl. XXXVI, fig. 2) is very similar to that illustrated by Sakisaka and Suehiro (1929) for *E. macrophylla* and that it would be difficult to distinguish between the "star" hair of *E. multiflora* as illustrated by Sakisaka and Suehiro and that of *Shepherdia argentea* (fig. 6). There is a striking similarity between the peltate hair of *S. canadensis* (fig. 1) and that of *Hippophaë rhamnoides* (Haberlandt, 1884).

The peltate hairs of *Shepherdia canadensis* (fig. 1) are much larger and the shield is made up of many more cells than is the case with the hairs of the other species examined (figs. 2, 3). These hairs are reddish-brown, and because of the long central stalks which raise the shields above the epidermis, they give a brown, scurfy appearance to the stem and leaves. The peltate hairs of *Shepherdia argentea* (figs. 3, 6) and of *Elaeagnus angustifolia* (fig. 2) are very similar to each other, have short stalks, and are silvery gray, giving to the foliage and young stems a smooth, grayish appearance.

The stellate hairs of *Shepherdia canadensis*, which seem to be limited to the leaves, consist of long and relatively fewer (usually eight to sixteen) ray cells than are to be found in the peltate hairs. The greater portion of each cell is free at the margin. The more or less "cottony" appearance of the lower surfaces of the leaves is due to the presence of mats of these hairs. In *Elaeagnus angustifolia* the stellate hairs (fig. 5) are much larger than the peltate hairs (fig. 2) and have more ray cells than those of *Shepherdia canadensis*. These are likewise limited to the leaves. An examination of the hairs on the leaves of *Shepherdia argentea* revealed the presence of two types of peltate hairs, a peltate hair (fig. 3) similar to that found in *Elaeagnus angustifolia* and a more or less rounded peltate hair whose ray cells are united

for only half their length (fig. 6). This latter type of hair seems to be limited to the leaves.

The stellate hairs (fig. 4) occur sparingly upon the upper surface of the leaf of *Shepherdia canadensis* and there is a dense mat of them on the lower surface, while an occasional stellate hair is to be found on the petioles. The stellate type is totally lacking on the bud scales and young stems. The peltate hairs are not present on the upper surface of the leaf. They are to be found along the veins on the lower surface of the leaf and are much more abundant than the stellate type on the petiole. Dense mats of the peltate hairs cover the bud scales and young stems. The hairs remain on the buds and young shoots until the following spring when they begin to drop away, and little trace of them is to be found on the two- and three-year-old twigs.

The shield of a peltate hair develops from a single epidermal cell, whereas the stalk develops from the hypodermal cells just below the epidermal hair initial and from the epidermal cells immediately surrounding it. The epidermal cell which is to develop into a shield enlarges somewhat (figs. 7, 14) and is easily distinguished from the surrounding epidermal cells by its thicker wall, more dense cytoplasm, and somewhat larger nucleus. By a division of this cell in a plane perpendicular to the surface of the epidermis, two thick-walled cells are formed (figs. 8, 15). These two cells elongate so that they project above the epidermis, and then both divide vertically in a plane at right angles to that of the first division, thus forming a four-celled shield (fig. 9). Each of the four cells now present divides a number of times vertically in planes that form acute angles with radii of the shield; the radiating partition walls thus extend from the central region of the shield but not from its exact center (figs. 10, 11, 12). Young hairs of *Shepherdia canadensis* consisting of 8, 12, or 16 cells are very numerous on the young stems. By the time the 8-celled stage is reached, the greater portion of each of the cells making up the shield protrudes above the epidermis, and these cells begin to elongate laterally from the central axis (figs. 16, 17). As the number of cells in the shield increases, each cell elongates still further, the diameter of the shield thus steadily increasing (figs. 18, 19).

While the elongation of the radiating cells is taking place, the nucleus of each cell migrates toward the outer margin of the shield. This migration is evident first in the 16-celled stage of the shield (fig. 12), and in the older growing shields the nuclei are always to be found near the marginal ends of the cells (figs. 18, 19).

With the increase in the size of the shield, the inequality in size of the cells arising from the division of a single radiating cell is very evident. One of the two daughter cells arising from a single cell of an eight-celled shield is somewhat larger than the other (fig. 10 *A*, *B*). In a shield consisting of 64 or more cells, the plane of division of a cell occurs at such an angle to the radius of the shield that the marginal cell which is cut off is about half as long as the parent cell (fig. 13). These marginal cells elongate and by con-

tinued divisions the shield enlarges until it contains perhaps 300 or more radially elongated cells. In the young shields the cells show various stages in nuclear and cell division. Bachmann (1886), describing the peltate hairs of the Elaeagnaceae, reported that the radiating cells do not all extend from the center, but that often a ray cell that arises at the middle point develops two or three cells so that a branched system of rays is formed.

The first evidence of the formation of a stalk occurs shortly after the shield has reached the 16-celled stage (fig. 19). The hypodermal cells beneath the central portion of the shield elongate somewhat and then divide by transverse walls. These new cells by continued growth push the central portion of the shield above the surface (figs. 19, 20) and hypodermal cells are forced into the aperture thus left in the epidermis. Both the hypodermal and the adjacent epidermal cells now divide (figs. 20, 21, 22) to form the multiseriate stalk which raises the shield above the epidermis. When the stalk is mature, it consists of a central core of hypodermal cells surrounded by a single layer of epidermal cells. Solereder (1908) reported that the cells of the stalk of a peltate hair in *S. canadensis* immediately below the terminal portion of the hair are short and have yellow walls. There is no evidence, in the material studied, of such short cells; the cells below the shield are on the contrary considerably longer than those that make up the remainder of the stalk (figs. 23, 24).

The method of formation of peltate hairs here described differs materially from that described for similar hairs by previous investigators. Schacht (1852, 1856) found that the scales of the Bromeliaceae are hairs whose stalks have not lengthened and whose disks are developed by growth and divisions of a single cell. Groenland (1855) in his study of the development of the glandular (peltate) hairs of *Pinguicula vulgaris*, found that an enlarged epidermal cell, by a horizontal division, divides to form an upper and a lower cell. The stalk develops from the lower and the cap from the upper cell. By vertical divisions the cap cell divides to form an umbrella-like shield. Chatin (1855) noted a similar type of development in the formation of the *cysties* on the lower surface of the leaf of *Callitriche*. Prillieux (1856) found that the peltate hairs of the 23 species of Oleaceae studied by him have a similar early development. He described the first appearance of the hair as an epidermal cell that protrudes above its neighbors. By a transverse division of this cell a primary cap cell and a stalk cell are formed. The cap cell is divided vertically, and then each daughter cell is divided in a vertical plane at right angles to the plane of the first division thus forming a four-celled cap. The hairs of all the Oleaceae are similar in their development to this point. The mature hairs vary in number of cells in the cap, from those of *Jasminum officinale* with four cells to those of *Olea europaea* with 32. When the cap is composed of more than a score of radiating cells it loses its disk-like form, each cell develops freely and irregularly at its extremity, and thus the cap takes on the form of a star. After having studied the

development of the hairs of the Oleaceae and having examined preparations showing the development of the hairs of *Pinguicula* and *Callitriche*, Prillieux concluded that all peltate hairs are developed in a similar manner.

The cells of the shield of the mature hair of *Shepherdia canadensis* are thick-walled and reddish-brown. The cytoplasm and nucleus of each cell has disintegrated. The elongated cells of the stalk just beneath the shield likewise become thick-walled, their protoplasm disintegrates, and their walls take on the color characteristic of the shield. The cells of the remainder of the stalk remain thin-walled and their protoplasm persists during most of the current growing season, but they ultimately become rusty brown.

It has been suggested that the mat formed of such hairs protects the plant against loss of water (Sakisaka and Suehiro, 1929). Eames and MacDaniels (1925) say that stellate, peltate, and other types of matted epidermal hairs form a dead air space next the epidermis wherein air is maintained at a relative humidity approaching that on the inside of the leaf, thus preventing water loss to the plant. Sayre (1920), after making a comparative study of the loss of water from normal plants of *Verbascum Thapsus* and from plants having the epidermal hairs removed, concludes that the value of the mat of hair as a protection against loss of water is negligible.

Summary

The peltate and stellate hairs in *Shepherdia canadensis*, *S. argentea*, and *Elaeagnus angustifolia* were found to be similar in size and shape, and within each species to be almost constant in these respects.

The hairs of *Shepherdia canadensis* are described in detail and compared as to structure and distribution with those of the other two species.

The development of these hairs is described for *S. canadensis*. The shield arises from a single epidermal cell and the stalk from adjacent epidermal and hypodermal cells. The hairs of this species were found to differ in development in several respects from similar structures in other families.

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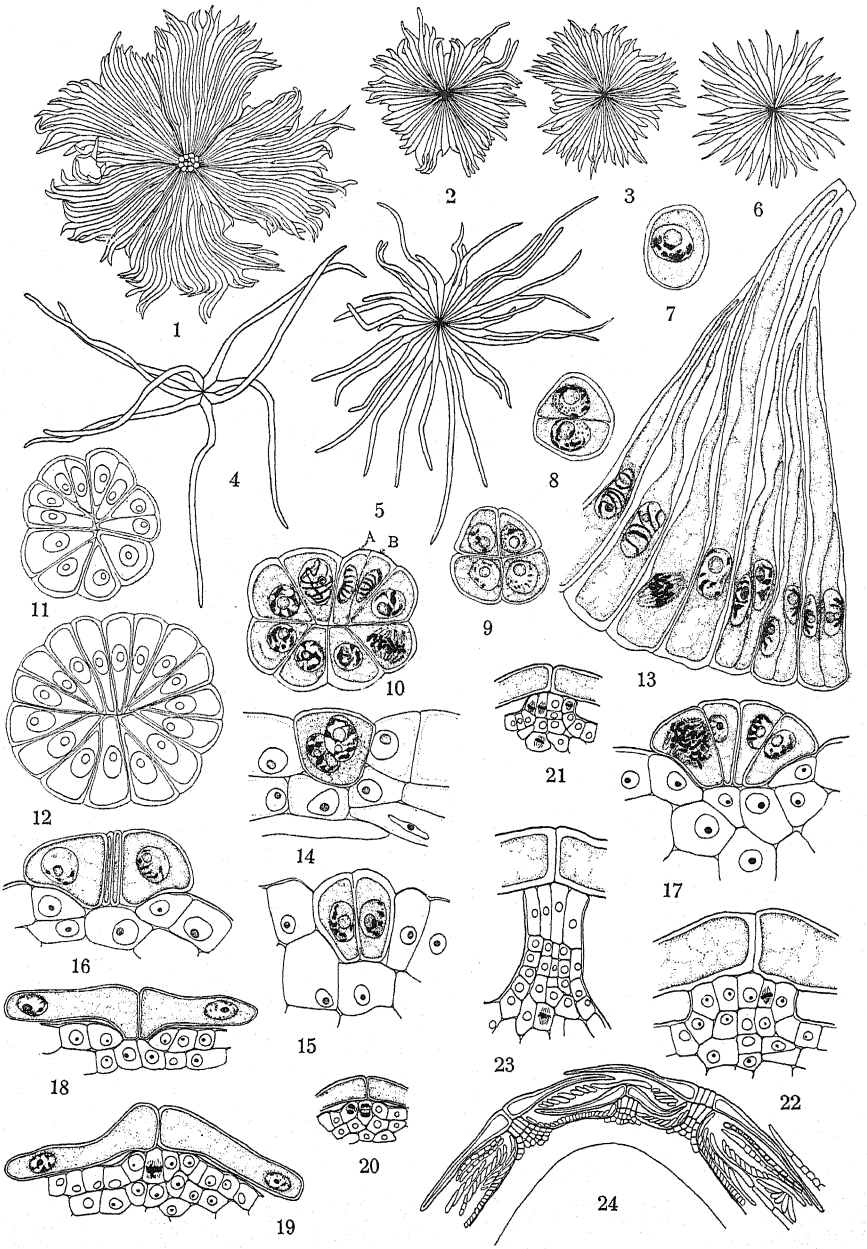
EXPLANATION OF PLATE XXXVI

FIG. 1. Peltate hair of *Shepherdia canadensis*, lower surface. × 70.
FIG. 2. Peltate hair of *Elaeagnus angustifolia*, upper surface. × 70.
FIG. 3. Peltate hair of *Shepherdia argentea*. × 70.
FIG. 4. Stellate hair from leaf of *S. canadensis*. × 70.
FIG. 5. Stellate hair from leaf of *Elaeagnus angustifolia*. × 70.
FIG. 6. Peltate hair from leaf of *Shepherdia argentea*. × 70.
FIG. 7. Single-celled epidermal peltate hair initial of *S. canadensis*. × 620.
FIG. 8. Two-celled stage of shield. × 620.
FIG. 9. Four-celled stage of shield. × 620.
FIG. 10. Nine-celled stage of shield. × 620.
FIG. 11. Twelve-celled stage of shield. × 620.
FIG. 12. Sixteen-celled stage of shield. × 320.
FIG. 13. Portion of an older shield showing the marginal nuclei and the formation of cell plates at an angle to the radius of the shield. × 620.
FIG. 14. Vertical section through epidermal hair initial. Two nuclei are present but cell wall has not yet been formed between them. × 620.
FIG. 15. Section through two-celled hair. × 620.
FIGS. 16, 17. Sections through older stages. Stalk has not yet begun to form. × 620.
FIG. 18. Section through older hair showing the nuclei toward the margin of the shield and an early stage in the development of the stalk. × 320.
FIG. 19. Section through a still older hair showing an early stage in the development of the stalk. × 320.
FIGS. 20, 21, 23. Stages in the development of the stalk. × 200.
FIG. 22. Vertical section through young stalk. × 320.
FIG. 24. A vertical section through a portion of a bud scale showing the mat of epidermal hairs. × 70.



COOPER: PELTATE HAIRS

THE CHROMOSOMES OF *SHEPHERDIA CANADENSIS*[1]

D. C. COOPER

(Received for publication December 3, 1931)

A study of the pollen mother cells of *Shepherdia canadensis* (L.) Nutt. was undertaken to determine the number of chromosomes and to detect, if possible, any morphological differences between the members of a pair of chromosomes that might be associated with the dioecious condition of this species. Sinotô (1929) and Miss Lindsay (1930) have made extensive studies of the chromosomes in dioecious plants. Both authors include lists of dioecious angiosperms in which heterochromosomes have been found and a list of species in which investigation has failed to reveal heterochromosomes in the sexes examined. No study has, thus far, been made of the chromosomes of *S. canadensis*.

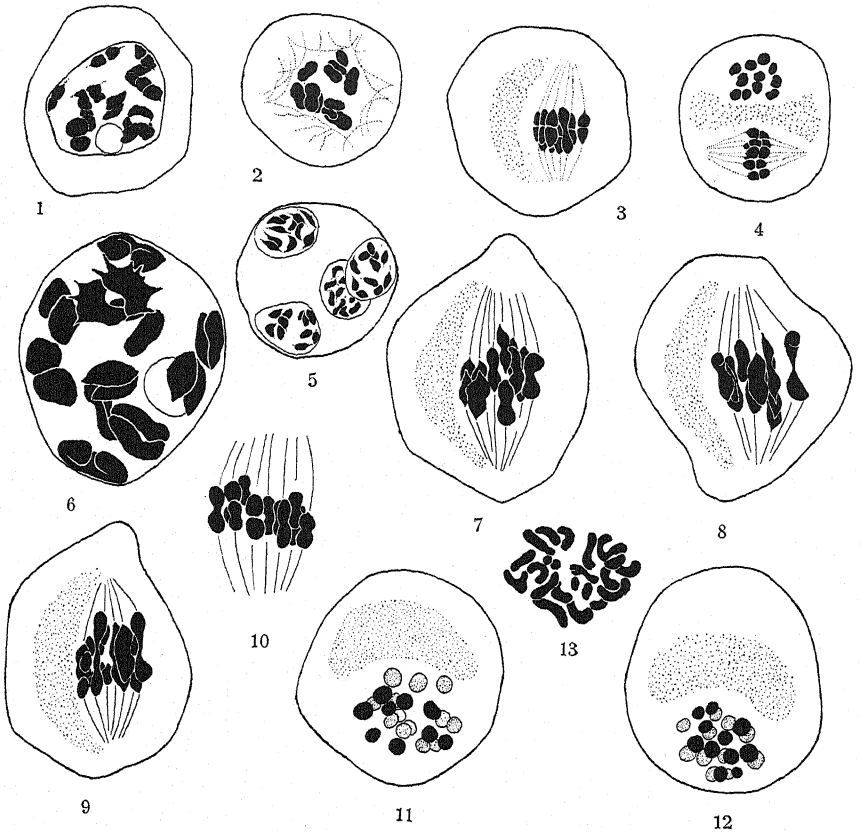
Staminate buds of this plant were obtained during the springs of 1929 and 1930 from plants growing near the Biology Building at the University Wisconsin. Buds of various sizes were fixed in Flemming's medium and Carnoy's solutions. The Carnoy's solution was made up as follows: 95% alcohol, 3 parts, chloroform 1 part and glacial acetic acid 1 part. Some of this material was cut in paraffin, and, in the case of that fixed in Carnoy's solution, the stamens were dissected from the buds and crushed in a drop of aceto-carmine. It was found to be extremely difficult to section the material imbedded in paraffin as the buds are covered with a dense mat of peltate hairs. Division figures are readily obtained in the microspore mother cells that are to be found in young, well-developed buds on which the peltate hairs are, as yet, silvery white. Tetrads are to be found in those slightly older buds on which the hairs are beginning to turn to their characteristic rusty-brown color.

The flower buds of *S. canadensis* appear in the axils of the young leaves shortly after the dropping of the matured flowers. The pollen mother cells pass through the reduction divisions and the pollen grains are formed during the first part of June. This pollen is not shed until the early part of the following April.

The number of chromosomes has heretofore been determined in only two species of the Elaeagnaceae. According to Miss Gaiser (1930), Sobolewska (1926) found the haploid number of chromosomes to be six in *Elaeagnus angustifolia* and ten in *Hippophaë rhamnoides*. In *Shepherdia canadensis* the writer has found the haploid number of chromosomes to be eleven, the diploid number 22.

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin No. 127. Published with the approval of the director of the station.

Aside from the difficulty of cutting the buds in paraffin, the pollen mother cells offer excellent material for study. At diakinesis the eleven chromosome bivalents vary considerably in size and are well separated so that they may easily be counted (text figs. 1, 6). Although O- and V-shaped figures



TEXT FIGS. 1–13. FIG. 1. Pollen mother cell of *Shepherdia canadensis* at diakinesis. × 1030. FIG. 2. Cell with multipolar spindle. × 1030. FIG. 3. The chromosomes at metaphase. × 1030. FIG. 4. Equatorial plates, homoeotypic division. × 1030. FIG. 5. Pollen mother cell with four granddaughter nuclei. × 1030. FIG. 6. Nucleus of a pollen mother cell at diakinesis. × 1940. FIGS. 7, 8, 10. Lateral views of heterotypic equatorial plates. × 1940. FIG. 9. Late metaphase. × 1940. FIGS. 11, 12. Polar views of heterotypic equatorial plates. × 1940. FIG. 13. Polar view of a somatic equatorial plate. × 1940.

are frequent, the longer chromosomes usually appear as thick rods. A large O-shaped pair is particularly prominent at this stage. In no case was it possible to find at diakinesis a bivalent made up of two unequal chromosomes.

Toward the end of diakinesis, the chromosomes tend to mass together in the central part of the shrinking nucleus. The nucleole disappears, and at

about the same time it becomes impossible to distinguish the nuclear membrane. Shortly after the disappearance of this membrane, from several points in the cytoplasm, just outside the nuclear cavity, fibers appear to extend toward the chromosomes (text fig. 2). While this multipolar spindle is developing, the members of each bivalent become closely appressed so that it is difficult to distinguish the individual chromosomes.

The division figures examined show a regular separation of equal chromosome pairs on both the heterotypic and homoeotypic spindles (text figs. 3, 4). The heterotypic spindle occupies a position toward one side of the cell. There is a densely granular mass of cytoplasm on that side of this spindle toward the center of the cell. During the homoeotypic division this granular mass occupies a broad zone midway between the two spindles (text fig. 4).

The somewhat rounded chromosomes are so compacted upon the heterotypic equatorial plate that it is almost impossible to count them with certainty in lateral view. Particular attention was given to the stages of separation of the homologous chromosomes (text figs. 3, 7–10). There was no evidence of a heteromorphic pair of chromosomes in the many figures examined. In late metaphase figures, certain of the chromosome pairs are conspicuous in that the constituent chromosomes adhere to each other as they move toward opposite poles and form a dumb-bell shaped figure before splitting (text fig. 9). The number of chromosome pairs is clearly eleven, as shown in polar views of equatorial plates of either division (text figs. 4, 11, 12). The homoeotypic devision seems to proceed regularly and four granddaughter nuclei are formed near the plasma membrane of the mother cell (text fig. 5). Chromosome counts were made of a number of polar views of equatorial plates in somatic cells (text fig. 13), and the diploid number was found to be 22.

Summary

1. The haploid number of chromosomes in *Shepherdia canadensis* is eleven, the diploid number is 22.

2. No evidence of a heteromorphic pair of chromosomes was found at any stage in the reduction division in the pollen mother cells.

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THICKNESS OF CUTICLE IN CRANBERRY FRUITS

NEIL E. STEVENS

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Records of thickness of cuticle in the fruits of even our cultivated plants are so rare in botanical literature that it seems worth while to publish the measurements of a number of varieties of the cultivated cranberry, which show a rather constant difference in the thickness of the cuticle in different varieties and a decided difference in a single variety in different years. The measurements which are summarized in table 1 were made during the month of November in 1929, 1930, and 1931.

The so called varieties listed in the table are all selections of wild vines of *Vaccinium macrocarpon* growing in eastern Massachusetts. Although many of them have been cultivated for several decades, not all are officially recognized as horticultural varieties, nor have the names used been so recognized. It is possible that in some cases a single variety appears under two names. However, in the case of varieties of uncertain identity, care was used to get the fruit from the same bog each year. The berries measured were all selected by H. J. Franklin or J. L. Kelley as being "true to name." It is thus reasonably certain that the same strain has been used under the same name in successive years.

The measurements published are averages of at least 10 individual measurements made in transverse sections taken approximately midway between the two ends of the berry. Sections from three or four different berries were used in all cases. During the early part of the work in 1929 much larger numbers of measurements were made, 300 in one case, but there was so little variation that this seemed unnecessary. During 1929 measurements were made in both cross and longitudinal sections but this also proved unnecessary. The thickness indicated includes, of course, not only the cuticle but the cell wall since the measurements were made from the outer edge of the cavity of the epidermal cell to the outer edge of the cuticle. Most of the measurements were made by Miss M. S. Wilcox and practically all were made with hand cut sections.

Inspection of table 1 shows that the thickness of cuticle ranges from 8.7 to 13.7 microns. That this difference is obviously not correlated with the size of the fruit was shown in some detail for the crop of 1929.[1] Very thick cuticle is found in some of the smaller varieties such as Bugle, while relatively thin cuticle is found in some of the large varieties such as MacFarlin. In all the varieties studied the cuticle was thicker in 1929 than in

[1] **Stevens, N. E.** Thickness of cuticle in cranberry varieties. Proc. Amer. Cranberry Growers Assoc. 61: 8–13. 1930.

1930 or 1931. This difference is so great, 20 percent or more between 1929 and 1930 in eleven of the varieties, as to exclude the possibility of observational error. Among the varieties measured in 1929, only the Early Black had a cuticle less than ten microns in thickness. In 1930, when the same varieties were measured, fourteen had a cuticle less than ten microns thick.

TABLE 1. *Thickness in Microns of Cuticle of Cranberry Fruits Grown in Massachusetts, 1929–1931*

Variety	1929	1930	1931
Early Black	9.9	8.7	9.0
Perry red	10.2	9.7	9.8
Horseneck	10.7	—	9.1
Klondyke	10.9	—	9.6
Rhode Island	10.9	9.9	9.9
McFarlin	11.1	9.4	9.9
Wales Henry	11.1	9.8	10.3
Kelley	11.2	9.4	9.7
Middleboro	11.3	9.6	9.9
Aviator	11.4	—	10.2
Holliston	11.4	9.7	10.9
Nova Scotia	11.4	—	10.3
Shurtleff	11.4	9.8	10.2
Smalley Howes	11.4	—	10.3
Buckelow	11.6	9.9	10.0
Pride	11.6	9.9	10.1
Murdock	11.7	—	10.1
Gifford	11.8	—	9.9
New Town	11.8	—	10.1
Russel Bell	11.8	—	10.0
Atkins Seedling	12.0	9.8	9.9
Centerville	12.0	9.9	11.1
Berry	12.1	10.0	10.8
Stanley	12.1	—	11.2
Tom Howes	12.2	10.0	10.3
Champion	12.3	10.2	10.8
Howes	12.4	9.9	10.8
Indian Head	12.4	10.0	10.1
Black Veil	12.5	—	10.5
Centennial	12.5	9.6	9.8
Mathews	12.7	10.0	11.2
Smith	12.7	—	10.5
Shaws Success	13.1	10.3	11.1
Bugle	13.5	11.8	11.9
Paradise Meadow	13.7	10.5	10.6

A smaller but apparently constant difference appears between the fruit of 1930 and that of 1931. Of the varieties measured in both these years practically all showed somewhat thicker cuticle in 1931.

The Early Black which is the most important commercial variety in Massachusetts and perhaps the best adapted to Massachusetts conditions of any yet discovered has the thinnest cuticle of any of those measured. The second variety in importance in this region, the Howes, has a thick cuticle.

The writer is unable to trace any relation between thickness of cuticle and weather conditions in the region where the cranberries are grown. The

summer of 1930, when the cuticle was noticeably thinner than in 1929, was one of extreme drought, but in the absence of experimental evidence no causal relation can be maintained. Information on rainfall and its distribution as well as temperature in this area is included in table 2.

TABLE 2. *Climatological Data for East Wareham, Massachusetts*

	Mean Temperature			Total Precipitation			Days with .01 inch or More Precipitation		
	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.
1929.....	69	66	63	2.29	5.79	3.59	9	6	11
1930.....	70	68	67	2.72	2.43	0.36	8	11	4
1931.....	72	70	64	3.91	5.00	1.81	12	14	5

The variation in thickness of epidermis in the different years is apparently not correlated with the size of the fruit. In 1929 and 1930, the volume of berries of each variety was determined in samples of 100 berries taken from the same lots as those of which the epidermis was measured. From twenty-five such samples measured in 1929 and 1930, 13 were somewhat larger in 1930, 9 somewhat smaller, and 3 of the same size in both years, yet the thickness of the epidermis was uniformally less in 1930.

Within a variety and among the berries of a single crop there is, at least in some cases, some correlation between the size of berries and thickness of cuticle. In 1929 we selected from a large lot of Early Black berries grown in East Wareham, Massachusetts, 100 very small, 100 very large and 100 medium size berries. The actual average volume of these lots, expressed in cubic centimeters per berry, was 0.85, 1.15, and 1.70. One hundred measurements were made of cross sections and one hundred of longitudinal sections in berries from each lot. The average thickness of cuticle in the small berries was 9.35 microns, for the medium sized 9.9, and for the large 10.1. In this case, at least, the cuticle seems to be slightly thicker in the larger berries. Even the largest Early Black of this year, however, still had a thinner cuticle than the average of any other variety measured.

This study was undertaken in the hope of discovering some correlation between the thickness of cuticle and resistance to the attacks of decay-producing fungi. No such relation appears, however, either between varieties or between successive crops. The Early Black with the thinnest cuticle measured has in general good keeping quality. The MacFarlin which has a thin cuticle has the best keeping quality of any of the so-called fancy varieties. The Middleboro with approximately the same thickness of cuticle is generally recognized as having exceptionally poor keeping qualities.

There is no apparent relation between the thickness of cranberry cuticle and the keeping quality of the crop as a whole. In 1931, when the thickness

of the cuticle was intermediate between that of 1929 and 1930, the crop as a whole showed the poorest keeping quality of any on record.

Summary

The thickness of the cuticle in thirty-three varieties of cultivated cranberries from Massachusetts ranged from 9.9 to 13.7 in 1929.

In all cases, the thickness of the epidermis in a given variety was greater in 1929 than in 1930 or 1931.

This difference in the different crops does not appear to be correlated either with size of fruit or the keeping quality of the crop or with any obvious differences in weather conditions.

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FIVE NEW GRASSES FROM TEXAS

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In a collection of grasses made by J. A. Moore and J. A. Steyermark, from the Guadalupe Mountains, one species was found to be new. A second undescribed species has previously been included in *Trichachne saccharata* (Buckl.) Nash (*Valota saccharata* (Buckl.) Chase), but recent studies show that it is distinct. The other three were obtained in eastern Texas by W. A. Silveus.

Festuca ligulata Swallen, sp. nov.

Perennis; culmi erecti graciles, e basi decumbentes, 45–65 cm. alti; vaginae laeves vel scaberulae, glabrae; laminae 6–30 cm. longae, 1–2 mm. latae, scabrae; ligula 3–3.5 mm. longa; panicula 6–10 cm. longa, pauciflora, ramis 1 vel 2, scabris, adscendentibus vel divergentibus, e basi nudis; spiculae appressae, 2–3-florae, 6 mm. longae; glumae acutae vel subobtusae, scabrae; gluma prima 3.5 mm. longa, 1-nervo; gluma secunda 4 mm. longa, 3-nervis; lemmata 4 mm. longa, acuta vel subobtusa, scabra, sine aristis; palea lemmata aequans.

Perennial; culms slender, loosely tufted, erect from a decumbent often rhizome-like base, 45–65 cm. tall, scabrous below the panicle, otherwise glabrous or nearly so; sheaths smooth or scaberulous, glabrous; blades 6–20 cm. long, those of the innovations as much as 30 cm. long, 1–2 mm. wide, rather firm, scabrous on both surfaces, strongly nerved above; ligule hyaline, 3–3.5 mm. long; panicles 6–10 cm. long, the axis scabrous, the one or two branches stiffly ascending or spreading, few-flowered, naked below, scabrous; spikelets 6 mm. long, 2–3-flowered, appressed to the branches; glumes acute or subobtuse, scabrous, especially on the midnerve, the first 3.5 mm. long, 1-nerved, the second 4 mm. long, 3-nerved; lemmas 4 mm. long, acute or subobtuse, scabrous, obscurely nerved, awnless; palea equaling or slightly exceeding the lemma (text fig. 1).

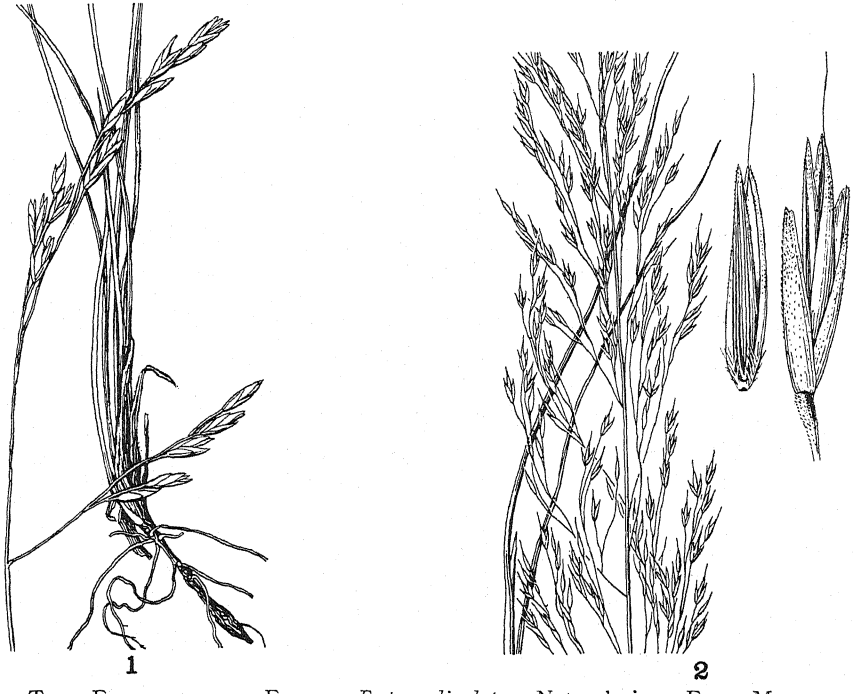
Type in the U. S. National Herbarium, no. 1501601, collected on moist shaded slopes along creek, upper McKittrick Canyon, Guadalupe Mountains, Culberson Country, Texas, alt. 1980 m., July 22, 1931, by J. A. Moore and J. A. Steyermark (no. 3576).

This species is related to *Festuca thurberi* Vasey, differing in its less densely tufted habit, more slender culms, smaller panicles of smaller, fewer flowered spikelets, and shorter, subobtuse lemmas.

Muhlenbergia involuta Swallen, sp. nov.

Culmi dense caespitosi, erecti, 65–135 cm. alti, scaberuli vel glabri; vaginae carinatae scabrae, internodiis longiores; laminae usque ad 25 cm. longae, involutae, scabrae; paniculae 28–40 cm. longae, graciles, ramis sub-

filiformibus adscendentibus, inferioribus ad 20 cm. longis; spiculae 3–3.5 mm. longae, pedicellis crassis strigosis; glumae 2–2.5 mm. longae acutae scabrae; lemma 3–3.5 mm. longum scabrum marginibus basi dense pubescentibus; arista 1.5–2 mm. longa, scabra.



TEXT FIGS. 1 AND 2. FIG. 1. *Festuca ligulata.* Natural size. From Moore and Steyermark 3576. FIG. 2. *Muhlenbergia involuta.* Natural size; spikelet and floret × 10. From Silveus 358.

Culms densely tufted, erect, 65–135 cm. tall, minutely strigose below the panicle, otherwise scaberulous or nearly smooth, glabrous; sheaths compressed-keeled, scabrous, much longer than the internodes; blades as much as 50 cm. long, wiry, involute, scabrous; ligule hyaline, 10 mm. long; panicles 28–40 cm. long, slender, erect, the subcapillary branches narrowly ascending or appressed, naked toward the base, the lower as much as 20 cm. long; spikelets 3–3.5 mm. long, the strigose pedicels thickened above; glumes subacute or somewhat erose, scabrous, 2–2.5 mm. long; lemmas 3–3.5 mm. long, scabrous, densely pubescent on the margins toward the very base, the minutely toothed apex awned from just below the teeth, the awn slender, scabrous 1.5–2 mm. long (text fig. 2).

Type in the U. S. National Herbarium, no. 1501594, collected in draws in hills, 20 miles northeast of San Antonio, October 10, 1931, by W. A. Silveus (no. 358).

Muhlenbergia involuta is related to *M. distichophylla* (Presl) Kunth but in that species the spikelets are only 1.5–2 mm. long, the awns are 10–15

mm. long, the blades are flat, as much as 5 mm. wide, and the sheaths bear auricles 2–3 cm. long. In both species the glumes are shorter than the floret.

Eragrostis silveana Swallen, sp. nov.

Perennis; culmi dense caespitosi, erecti, 40–50 cm. alti, glabri; vaginae glabrae; laminae usque ad 25 cm. longae, 3 mm. latae, attenuatae, glabrae; ligula 0.2–0.3 mm. longa; paniculae 25–35 cm. longae, ramis viscidis scabris adscendentibus vel divergentibus, basi nudis; spiculae 2.5–4 mm. longae, 4–8 florae; glumae 1 mm. longae, 1-nervo; lemmata 1.2–1.3 mm. longa, acuta, nervis lateralibus prominentibus.

Perennial, culms densely tufted, erect from a knotty base, 40–50 cm. tall, glabrous; sheaths longer than the internodes, smooth, glabrous; blades flat or drying loosely involute, as much as 25 cm. long, 3 mm. wide, attenuate to a fine point, smooth, glabrous; ligule minutely ciliate, 0.2–0.3 mm. long; panicles 25–35 cm. long, 10–15 cm. wide, partly inclosed in the upper sheaths, the viscid, scabrous branches stiffly ascending or spreading, naked at the base, the pulvini sparsely pubescent or pilose; spikelets purplish, 2.5–4 mm. long, 4–8 flowered, those of the branchlets on short, usually appressed pedicels; glumes 1 mm. long, or the first a little shorter, 1-nerved; lemmas 1.2–1.3 mm. long, acute, the lateral nerves prominent (text fig. 3).

TEXT FIG. 3. *Eragrostis silveana.* Natural size; spikelet × 10. From Silveus 360.

Type in the U. S. National Herbarium, no. 1501596, collected along railroad near Taft, Texas, October 1, 1931, by W. A. Silveus (no. 360).

This species is viscid as in *E. curtipedicellata* Buckl., but is distinguished by the more open slender panicle of long- pedicellate spikelets. The inclosed base of the panicle suggests *E. spectabilis* (Pursh) Steud. (*E. pectinacea* of manuals), but the panicle is not rigid, the spikelets are smaller, and the foliage is glabrous throughout.

E. silveana is named for Mr. W. A. Silveus, of San Antonio, who has contributed numerous collections to the National Herbarium.

Trichoneura elegans Swallen, sp. nov.

Annua; culmi erecti 35–110 cm. alti, glabri, infra paniculam scabri; vaginae carinatae, papillosae, scabrae, internodiis longiores; laminae 5–25 cm. longae, 2–7 mm. latae, scabrae, pungentes; ligula 2 mm. longa, truncata; paniculae densae, 15–18 cm. longae, 5 cm. latae, spicis 15–20, adscendentibus, inferioribus ad 8 cm. longis; spiculae, 9–10 mm. longae, 7–8-florae, pedicellis scabris; glumae acuminatae, spiculam aequantes; lemma inferior 5.5 mm. longum, infra glabrum, supra scabrum, obtusum, callo barbato, marginibus ciliatis; palea acuta, dentata, scabra; rachilla basi barbata.

Annual; culms erect, 35–110 cm. tall, strigose below the panicle, otherwise glabrous; sheaths mostly longer than the internodes, more or less distinctly keeled, papillose, scabrous; blades 5–25 cm. long, 2–7 mm. wide, narrower at the base than the summit of the sheath, pungently pointed, scabrous on both surfaces; ligule 2 mm. long, membranaceous, truncate; panicles finally exserted, 15–18 cm. long, 5 cm. wide at maturity, densely flowered, the axis strigose; racemes 15–20, approximate, narrowly ascending to somewhat spreading, the lower as much as 8 cm. long, the rachis 3-angled, scabrous on the angles; spikelets 9–10 mm. long on short strigose pedicels, 7–8 flowered, the lowest floret sterile; glumes equaling the florets, acuminate, awn-pointed, 1-nerved, scabrous; lemmas 3-nerved, the lateral nerves marginal, glabrous below, scabrous toward the obtuse minutely two-lobed summit, awned from between the lobes, the awns not more than 0.5 mm. long, the margins conspicuously ciliate on the lower half or two thirds with spreading hairs as much as 1 mm. long, the flattened callus bearded on the margins, the lower 5.5 mm. long, the others gradually shorter; rachilla joint densely bearded at the base, otherwise glabrous; palea acute, 2-toothed a little shorter than the lemma, scabrous on the back and keels (text fig. 4).

Late in the season the culms branch at the upper nodes and send up numerous small culms from the base, often as short as 10 cm. long, the panicles small and densely flowered.

Type in the U. S. National Herbarium, no. 1501591, collected in sandy land south of Devine, Texas, September 26, 1931, by W. A. Silveus (no. 343).

This is the first species of this African and South American genus to be found in the United States. *Trichoneura* Anderss. is allied to *Leptochloa*, differing in the much larger spikelets, the acuminate or awn-pointed glumes which equal or exceed the spikelet, the flattened bearded callus, and the bearded base of the rachilla-joint.

TEXT FIG. 4. *Trichoneura elegans*. Natural size; spikelet and floret × 10. From Silveus 343.

TEXT FIG. 5. *Trichachne patens*. Natural size; spikelet and floret × 10. From Reed 11.

Trichachne patens Swallen, sp. nov.

Perennis; culmi erecti e basi geniculati, 40–90 cm. alti, glabri; vaginae internodiis longiores, superiores papilloso-pilosae, infimae dense pubescentes; laminae 5–15 cm. longae, 1–4 mm. latae, scabrae et pilosae; ligula 2.5–3 mm. longa; panicula 10–18 cm. longa, ramis scabris, rigidis, adscendentibus vel divergentibus; spiculae 4–5 mm. longae; gluma prima 0.3–0.5 mm. longa; gluma secunda 3 mm. longa 3-nervis; lemma sterile 3 mm. longum, 5-nervis; lemma fertile 3 mm. longum, acutum, minute puncticulato-scabrum.

Perennial; culms tufted, erect, or sometimes geniculate at the lower nodes, 40–90 cm. tall, smooth, glabrous; sheaths longer than the internodes, sparsely to rather densely papillose-pilose with spreading or retrorse hairs, the lowermost densely velvety pubescent; blades 5–15 cm. long, 1–4 mm. wide (mostly 2 mm. wide), scabrous, especially the margins, and usually sparsely pilose as well; ligule 2.5–3 mm. long, membranaceous; panicles 10–18 cm. long, the slender scabrous branches stiffly ascending when young, spreading when mature, comparatively few-flowered; spikelets distant, densely white silky, 4–5 mm. long including the hairs, early deciduous, single or paired, when in pairs one pedicel 1–2 mm. long, the other 3–5 mm. long, that of the terminal spikelet 10–12 mm. long; first glume 0.3–0.5 mm. long; second glume and sterile lemma 3 mm. long, equaling the fruit, the glume narrow, 3-nerved, exposing a part of the fertile lemma, hairy on the nerves and internerves, the sterile lemma broader, 5–nerved, covering the fruit, hairy only on the marginal nerves; fruit 3 mm. long, 0.7–0.8 mm. wide, oblong-lanceolate, acute, minutely striate-roughened, dark brown (text fig. 5).

Type in the U. S. National Herbarium, no. 927233, collected on dry prairie near Lake Mitchell, San Antonio, Texas, June 18–24, 1910, by A. S. Hitchcock (no. 5328) ["Amer. Gr. Nat. Herb. no. 294."].

Dry fields, prairies, and roadsides, Texas. San Antonio, *Hitchcock* 5328, *Swallen* 1774, *Silveus* 153; Kingsville, *Reed* 11, *Piper* in 1906; Beeville, *Swallen* 1860; Encinal, *Griffiths* 6379; Moore, *Palmer* 33844a; without locality, *Lindheimer* 723.

This species has previously been referred to *Trichachne saccharata* (Buckl.) Nash. In the field, however, it has a quite distinct appearance, the panicle branches being stiffly ascending or spreading instead of appressed as in *T. saccharata*. The spikelets differ in being less densely hairy and in the oblong lanceolate fruit, 0.7–0.8 mm. wide, gradually narrowed to an acute, indurate tip, those of *T. saccharata* being obovate, 1–1.1 mm. wide, rather abruptly narrowed to the longer scarcely indurate point. In both species the fruit is dark brown and minutely striate-roughened.

T. insularis (L.) Nees is readily distinguished from *T. patens* by the fruit which is 4 mm. long, not abruptly pointed, and by the usually larger and denser, tawny-colored panicles with appressed or narrowly ascending branches.

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ANATOMICAL AND DEVELOPMENTAL STUDIES OF THE FOLIAR EMBRYOS OF *BRYOPHYLLUM CALYCINUM*[1]

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INTRODUCTION

Morphological and anatomical studies of the leaves of *Bryophyllum calycinum* seem to have been somewhat neglected although much experimental physiological work has been done upon this plant. The ease with which its leaves give rise to plantlets explains its frequent use in so-called "regeneration" studies. Howe (6) and Beals (1) have recorded morphological and anatomical data concerning these plantlets. Beals (1, p. 375) states that "regenerated" structures in *Bryophyllum* arise from the phloem cells of the vein near the notch of the leaf. She mentions in no connection any observations of the dormant meristem referred to by former writers, *e.g.*, Sachs (17), Kerner and Oliver (9), and Kupfer (10), and which is normally present in the notches of all foliage leaves 2 mm. or more in length. Yet it seems obvious from her drawings (1, Pl. 15) that she was dealing with normal, adult leaves of the plant. Nor is her conclusion verified by the work of Howe who states (6, p. 387) that the shoot primordium of the plantlet arises exogenously in the leaf notch from latent meristem and that the roots arise endogenously from a point which she did not determine. Howe recognizes the group of meristematic cells in the leaf notches of "very young leaves" although she does not give the exact size of such leaves. She also states (6, p. 389) that the young "bud" is definitely organized on an adult attached leaf and that the growth of well developed plantlets is possible upon such a leaf.

Theories as to the causal factors involved in plantlet development upon the leaves of *Bryophyllum* have been presented by Sachs (17), Goebel (5), McCallum (13, 14), Loeb (12), Child and Bellamy (4), Reed (16), Kakesita (8), and Mehrlich (15), but they have dealt very little with the morpho-

[1] Contribution from the Botanical Laboratory of the University of Oklahoma, N. S. no. 15.

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logical nature or the origin of the "regenerated" structures. On the other hand Kupfer (10) and Isbell (7) have pointed out that since meristem is present in the leaf notches of *Bryophyllum*, plantlet development therefrom cannot be properly termed "regeneration." In view of such conflicting interpretations of the data and of the meaning of the term "regeneration," a complete reëxamination of the problem would seem desirable.

The present work, therefore, is an attempt to relate plantlet production in *Bryophyllum* to the normal life history of the organism through a study of leaf development and structure. The results agree in general with those obtained by Howe, though several points of difference will appear in further discussion. No attempt has been made to investigate the causal factors involved in plantlet production since, in the writer's opinion, more information is first of all needed regarding the complete developmental history of the plantlets in relation to the ontogeny and structure of the leaf which produces them.

Materials and Methods

Two large plants of *Bryophyllum calycinum* growing in the greenhouse of the Botany Department at the University of Oklahoma were used as a source of material. Leaves placed upon moist sand, leaves hung with the apices dipping in water, and small leaf segments, each including a notch and its associated dormant meristem, furnished an abundance of plantlets for study. In preparing slides for histological study, the usual method was employed as is outlined by Chamberlain (3). Safranin followed by Orange G was used successfully on paraffin sections of the succulent leaf tissue.

For the studies of venation, leaves were placed in a saturated aqueous solution of chloral hydrate to which a few drops of 1 percent safranin in absolute alcohol were added. A dissecting binocular was employed for venation studies, early stages of leaf development, and early stages of plantlet development.

In these studies sections were obtained from leaves as small as 2 mm. in length in which notches had just started to develop. The entire series investigated ranged from this size up to the adult leaves which were 10 cm. or more in length. Both the thin "summer" leaves and the thicker "winter" leaves[2] were studied, for there seemed to be some difference in the behavior of the two types.

Results

Organization of the Shoot System

The stem of *Bryophyllum* is generally unbranched, although development of axillary buds may occasionally occur and thus produce short branches. Since the lower leaves are continually abscissing, the lower portion of the

[2] Leaves produced in the late spring and throughout the summer were considerably thinner than those produced in the winter.

stem is left naked. Axillary bud development rarely occurs on this leafless part of the stem.

The leaves are opposite and decussate, although the first pair of leaves produced by a young plant may be displaced 2 or 3 mm. from each other. In some instances one may be abortive or entirely lacking. Foliage leaves formed early in the development of the shoot are always simple but later ones are compound with either three or five leaflets pinnately arranged. There seems to be little order in the sequence of appearance of these compound forms; ternate leaves may appear above and below those with five leaflets. Frequently teratological forms are produced in which there are two or four leaflets, the terminal one being united with one of the neighboring lateral ones. The simple leaves were generally employed throughout this study. However, leaflets of the compound forms seemed to be equally capable of producing plantlets.

The simple leaves and leaflets are ovate to elliptical in outline with a rounded base (Pl. XXXVII, fig. 3). The margin of normal foliage leaves is crenate, the number of sinuses or notches being 20 to 22 in old leaves. The first two or four leaves produced in the growth of a plantlet have few notches, sometimes only two or three (fig. 1). The bracts of the inflorescence also show a reduction in the number of sinuses as well as in size, the uppermost ones being reduced to a lanceolate structure with an entire margin (fig. 4).

Anatomy of the Leaf

An anatomical study of the foliage leaf of *Bryophyllum* indicates that it possesses a relatively simple structure (Pl. XXXVIII, fig. 14). Aside from the two epidermal layers and the ramifying veins, the bulk of the leaf is made up of large parenchymatous cells abundantly supplied with chloroplasts. There is no differentiation into palisade and spongy parenchyma.

The mesophyll of mature leaves consists of large, thin-walled cells which have no regular arrangement. This region is, however, about nine cells in width. The cells of this mesophyll are very closely packed, almost eliminating intercellular space. Small air chambers appear in the mesophyll near the stomata but are much reduced.

Investigations of "winter" and "summer" leaves disclosed that each type contained approximately the same number of cells in cross section but that the "winter" leaves measured 2500 micra in thickness and the thinner "summer" leaves only 560 micra. Individual cells of the thick leaves averaged 300 micra in diameter and cells of the thin type about 60 micra. Thus it is clear that the difference in leaf thickness is due to cell size and not to cell number.

A pigment, identified by the writer as one of the anthocyanins, is found scattered throughout the cells of the leaf, most abundantly just under the epidermis and in the notches of the older leaves.

Ontogeny of the Foliage Leaf

A study of leaf ontogeny revealed nothing strikingly unusual. The leaf primordia arise in decussate fashion as minute papillae upon the conical growing point (Pl. XXXVII, fig. 5). The plastochron in this case is quite long; thus considerable differentiation of tissues and maturity of one pair of leaves occur before the next pair appear on the growing point.

As the primordia develop, marginal growth becomes more pronounced and results in the formation of the two wings of the lamina. Coincident with the development of the wings of the lamina there is the appearance of crenations in the leaf margin which develop in a basipetal direction (fig. 6). It is clear that the crenations appear very early in leaf ontogeny, even upon leaves 2 or 3 mm. long (figs. 6 and 8). Continued expansion of the wings of the lamina and its rapid elongation bring the leaf blade into the adult condition (fig. 3). The petiole develops from the lower portion of the leaf primordium as a result of an increase in diameter and the failure of pronounced marginal growth, its demarcation at early stages being rather indefinite.

Origin of the Foliar Embryos

Serial sections through leaf primordia in which crenations have not appeared give no indication of meristem except at the margins where the wings of the lamina are being built up. Tangential sections (cut parallel with the ventral leaf surface) through very young leaves 2 or 3 mm. long, however, disclose a patch of meristem in the sinus of each crenation. Many such sections have yielded meristematic masses of various degrees of complexity or size, and a study of these shows that the development of the dormant meristem is clearly correlated with the development of the notch of the leaf.

In the early stages of formation, this meristem consists of only two or three tiers of cells (Pl. XXXVIII, fig. 9). As leaf growth continues, however, proliferation of meristem in the notches also continues so that in old leaves 6–8 cms. long a very complex aggregation of meristematic cells occurs (fig. 15). Even in old leaves the outermost layer of cells composing this meristematic mass is definitely meristematic in character and forms the dermatogen of future primordia if they develop. In the region of the notch there is an abrupt transition from the small, meristematic dermatogen cells to the large vacuolated cells of the leaf epidermis (fig. 15). This is in accord with the work of Howe (6), who concludes that the development of the "bud" is "exogenous."

Upon all fully matured leaves (6–8 cm. long) examined it was found that this meristem of the notch had developed a stem primordium and the first two leaf primordia (fig. 11). Though quite small, these leaf initials were usually evident. At this stage there was no indication of vascular connection of the notch meristem with the underlying vein. As a rule

further development of these primordia does not occur while the leaf is attached, though it is possible for attached leaves to bear plantlets with well developed shoots and roots. Such growth occurred upon plants which were regularly watered and in a moist atmosphere. Similar observations have been made by Howe (6), Braun (2), Reed (16), and Mehrlich (15).

Roots do not ordinarily make their appearance until the leaf has been detached from the plant and growth of the notch meristem stimulated. They are quickly organized, however, and become macroscopically evident within about four days. Sections through leaves that have been removed from the parent plant for a day or two reveal the organization of roots just below and lateral to the meristematic mass forming the shoot primordium (fig. 7). The young roots push their way out through the surrounding parenchyma cells, at the same time establishing vascular connection with the near-by vein. Coincident with this, mitotic activity has been renewed in the region of the shoot primordium, establishing vascular connection with the underlying vein. Thus a considerable mass of cells is concerned in the development of the new root and shoot. Other roots make their appearance soon after the first one, taking their origin from the same general region, *i.e.*, the active mass of cells just below the developing shoot.

It is this regular appearance of meristem in the notches of the leaves of *Bryophyllum* and its tendency to give rise to both shoot and root that seem to justify the use of the term "foliar embryo." The terms "bud," "foliar bud," and "epiphyllous bud" seem hardly applicable when one is dealing with rudiments of roots as well as of shoots. Howe (6) did not determine the exact origin of the first root but states (6, p. 388) that it arises "somewhere near the vein." When it is remembered that the meristem of the notch is, in mature leaves, a considerable mass of cells reaching nearly to the vein, it seems best to regard the first root at least as a fundamental part of that meristem, *i.e.*, the foliar embryo.

Development of Plantlets upon Leaves

The normal stimulus for plantlet development is detachment of the leaf from the plant, though a variety of stimuli have produced such results according to the work of Child and Bellamy (4), Reed (16), Kakesita (8), and Mehrlich (15). Kakesita's work indicates that the stimulus causing plantlet development upon detached leaves is the intramolecular or anaerobic respiration occurring there. He obtained plantlet development upon normal attached leaves by injecting into them the compounds known to be formed by intramolecular respiration, while controls injected with pure water showed no such development.

The recent work of Mehrlich casts much doubt upon the conclusions reached by Kakesita as well as those of other earlier workers. Mehrlich attributes the development of plantlets not to a single cause but rather to

the complex interaction of many factors. Though a rather unsatisfying one, such a position would seem to be best in view of the conflicting results of so many investigators.

It has been shown by other workers that leaves with the foliar embryos removed will exhibit no plantlet development. The writer found no exceptions to this observation. Yet it was thought that if foliar embryos were removed from very young leaves while attached to the plant, there might occur a replacement of these structures. A hot needle was used to destroy the foliar embryos on several attached leaves 1–3 cms. long. The plants to which these leaves were attached continued normal growth, yet there was no replacement of foliar embryos upon the leaves. Callus formation only was observed at the places of injury. In none of these cases were the ordinary tissues of the leaf seen to produce either roots or shoots. Their production in the case of *Bryophyllum* seems to be dependent entirely upon the foliar embryos, a fact again emphasizing the intimate relationship of the development of plantlets to the normal ontogeny of the foliage leaf.

The Apex Patch

A study of the venation of *Bryophyllum* leaves cleared in the manner already indicated reveals one of the most interesting problems of the present investigation, namely, the presence in each crenation of the leaf of a patch of cells completely surrounded by vascular elements. Their appearance under the dissecting binocular is that of dark patches or spots, more or less triangular in outline (fig. 2). These cell patches are to be distinguished from the foliar embryos which lie in the sinuses of the leaf. Tangential and cross sections through the leaf reveal that the cells of these peculiar patches are small and closely packed, possessing prominent nuclei (figs. 10 and 13). Anastomosing veins completely surround this mass of cells. In mature leaves, at least, there are several layers of parenchyma between this patch and the epidermis of the leaf. The youngest leaves examined possessed these structures in essentially the same form as found in adult leaves.

There is thus definite correlation between the appearance of these cell patches in the leaf and the development of notches (figs. 1 and 2). It is also of interest to note that these patches of meristematic-like cells have been found associated with the crenations of the floral bracts. Their presence in the apex of each crenation of normal foliage leaves and bracts has led the writer to apply the term "apex patch" to them in the absence of further knowledge concerning them.

Experiments were conducted to determine what role these apex patches might play in the development of the foliar embryos of the leaf. Their removal from the leaf did not inhibit plantlet development though it did seem to reduce somewhat the vigor of their growth. The severance of vascular connections by the razor may in this case explain the reduction of

vigor. Under no conditions was it possible to secure growth of any sort (except callus formation) from the cells of the apex patch, though they clearly appear to be meristematic in character. Their significance, if any, must constitute for the present an unsolved problem.

Discussion

That the production of foliar embryos is a perfectly normal process in the history of development of the leaf in *Bryophyllum* cannot be too strongly emphasized. Their presence is in no sense unusual from the standpoint of the life of this particular organism, however rare it may be within the plant kingdom as a whole. The results of the present study indicate that as the wings of the laminae expand there is a tendency for a small group of marginal meristem to be set off or left behind in the development of the notch. Although it has not been possible to trace the foliar embryos back to a one or two celled stage, it seems unlikely that they arise as direct descendants of dermatogen cells. In the very early stage of leaf development in which they are laid down, inner tiers of cells as well as the dermatogen itself are meristematic and probably enter into the formation of the meristematic mass (fig. 9). These cells remain in an embryonic condition despite the maturation and differentiation of the neighboring cells and, as the leaf matures, build up primordia which may remain dormant for a considerable period or may produce a new plant if the proper conditions are brought about.

In view of these facts it would seem that to apply the term "regeneration" to plantlet production upon the leaves of *Bryophyllum* would be to misuse it or at least to extend it to the point of vagueness. It is true that there exists a wide variety of opinions as to the use of the term "regeneration" among plants. Some would restrict the term to those cases where as a result of injury or loss organs are formed which are identical or nearly so with those removed, *e.g.*, Němec, Pfeffer, and Prantl. On the other hand, Vöchting, Goebel, Klebs, and Morgan use the term "regeneration" to include a much wider variety of phenomena, even the development of latent meristems present before injury or treatment. It would seem, as Isbell (7) points out, that a more consistent view is that expressed by Kupfer (10, p. 196) who says "regeneration ought to be limited to those cases in which an organ is formed, *de novo*, at a place or under conditions in which it would not normally be found." This definition clearly excludes all cases in which preformed rudiments can be detected. It is this distinction which seems to the writer most reasonable and most likely to meet with a certain measure of success when applied to the various examples of so-called regeneration in question. The writer thus suggests that the term "regeneration," at least with reference to plants, should be reserved for those cases in which new organs are formed, *de novo*, apart from the action of any preformed meristem and at places where they would not

normally be found in the life history of the organism. Under this interpretation it is clear that plantlets produced upon the leaves of *Bryophyllum* do not come within the category of regenerated structures.

Since the meristem in the notches of *Bryophyllum* leaves clearly forms a shoot primordium and since roots may also arise from the inner, basal part of this same cell mass, it seems that the condition may be more clearly expressed by the use of the term "foliar embryo" than by the term "bud." While it is evident that this foliar embryo may not be homologized with embryos of seeds which are the result of the fusion of gametes and the development of the zygote, the two structures are potentially the same and it is in this sense only that the term "embryo" is suggested to apply to the meristematic cell masses in the notches of *Bryophyllum* leaves. The analogy appears to be a safe one, especially when it is remembered that the foliar embryo furnishes the most effective means for both the natural and artificial propagation of *Bryophyllum*.

It is of interest to note in this connection what LeMaout and Decaisne (11, p. 708) say of the "buds" on the leaves of *Bryophyllum* "whose leaf produces buds furnished with root, stem, and leaves at the extremities of its lateral nerves. These buds, which spontaneously fall off, and root in the earth, may be likened to embryos that do not need to be fertilized before developing and the leaf of *Bryophyllum* may be regarded as an open carpel, on which the seeds have been developed by nutritive action alone. This fecundity of *Bryophyllum* completes the analogy between the true bud and the fertilized embryo." Although this early statement of the case shows clearly the influence of speculative philosophy, there is indeed a portion of truth contained therein.

Although it is impossible at present to draw any conclusion regarding the function or significance of the apex patches, their presence must be recognized and dealt with in the interpretation of the morphology of the *Bryophyllum* leaf. Their occurrence in at least one other species of the genus has recently been determined, namely, *Bryophyllum crenatum*. No account of such structures in leaves of other plants has been found, so at present they seem to represent an anomaly.

When the intimate relation of foliar embryos to the normal developmental history of the leaf of *Bryophyllum* is appreciated, it appears that the physiological work previously done attacks the problem from the wrong standpoint. In place of determining why plantlets develop upon detached or otherwise experimentally controlled leaves, it would rather be in order, as Mehrlich (15) intimates, to determine why plantlets *do not develop* upon normal, attached, actively functioning leaves. The meristematic cells destined from early leaf ontogeny for the production of root and shoot primordia are arrested in their development upon the normal foliage leaf. It seems that this arrest in normal development represents the significant problem to be studied from a "causal" standpoint.

Though it is clear that anatomical and developmental studies do not furnish explanations of *why* certain phenomena occur, it is equally clear that they furnish a very necessary *basis* for the explanations of cause. Whatever may be the nature of the stimulus or stimuli which either limit or encourage active division of the meristematic cells composing the foliar embryos found in *Bryophyllum*, their action must be conditioned upon the nature of the foliar embryo, its origin, and developmental history.[3]

Summary

1. The foliage leaves of *Bryophyllum calycinum* readily develop roots and shoots in their notches and present an interesting problem in so-called plant regeneration.

2. Foliage leaf primordia arise decussately at the growing point. Crenations which develop basipetally appear when the primordia are about 2 mm. long. The base of the primordium develops into the petiole.

3. The mesophyll of the leaf is composed of large, globose parenchyma with little intercellular space. No palisade and spongy layers are found.

4. Meristem cells appear in the leaf notches coincident with their formation and build up foliar embryos upon adult leaves. The foliar embryos early exhibit a shoot primordium and later may produce roots.

5. Foliar embryos occur in all notches of foliage leaves and in many bracts of the inflorescence.

6. Plantlet growth upon the leaf was secured only from uninjured foliar embryos. Roots arose from the inner portion of the foliar embryo and pushed their way through the surrounding parenchyma. Roots appeared in about four days. The shoot appeared shortly afterward, arising from the latent primordium.

7. A patch of meristem-like cells is early set apart in the apex of the leaf crenations. This apex patch, completely surrounded by vascular elements, is apparently unrelated to the foliar embryo of the notch. No primordia arose from the apex patch, nor did its absence or presence influence to any great extent the development of the foliar embryos.

8. The term "regeneration" does not seem to apply to the production of epiphyllous plantlets in *Bryophyllum* since latent meristem is always present in the leaf notches.

9. The real physiological problem seems to be the determination of the factors causing arrest of foliar embryo development rather than its initiation.

[3] Following the date of acceptance of this article for publication there appeared in the January number of this JOURNAL (Amer. Jour. Bot. 19: 32–40. 1932) a paper by Naylor upon *Bryophyllum* which agrees in many respects with the findings herein described. He does, however, accept the term "regeneration" as applied to *Bryophyllum* and also describes a "foot" in connection with the foliar meristems. A little earlier there appeared a preliminary announcement by the writer (Science **75**: 84–85. Jan. 15, 1932) of his work on *Bryophyllum*. This article stressed the importance of the regular appearance of the dormant meristem in the leaves, termed then "foliar embryos," and called attention to the difficulty of applying the term "regeneration" to this phenomenon.

The writer wishes to express his appreciation to Dr. A. S. Foster for his assistance and suggestions throughout the course of this study. Dr. R. B. Wylie has also made helpful suggestions.

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EXPLANATION OF PLATES

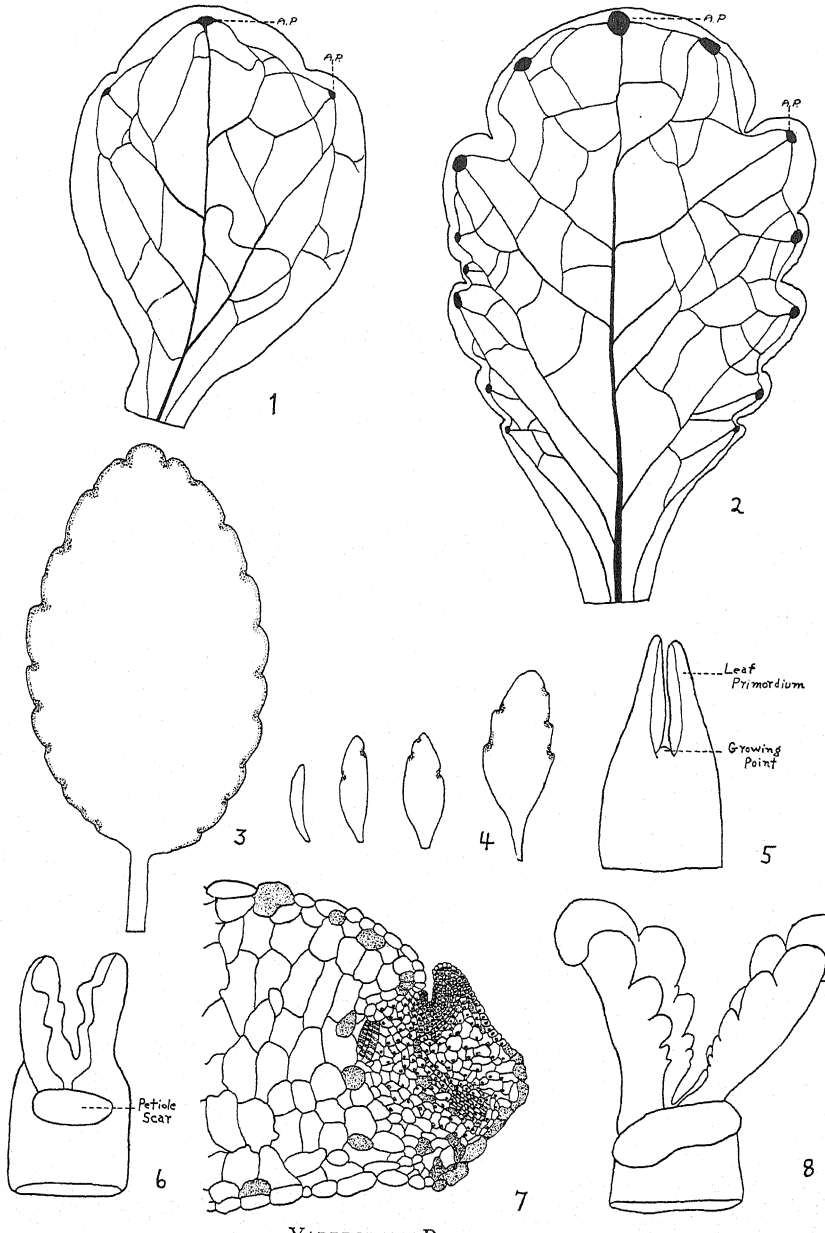
Cell contents are not shown except for anthocyanin pigment and nuclei of dormant meristem and growing points. Sketches of venation show only the most important veins of the leaf. On account of greater reduction than was intended, the magnification is slightly less than that indicated. The following abbreviations are employed throughout: *A.P.*, apex patch. *Ep.*, epidermis. *An.*, anthocyanin. *U.Ep.*, upper epidermis. *L.Ep.*, lower epidermis.

PLATE XXXVII

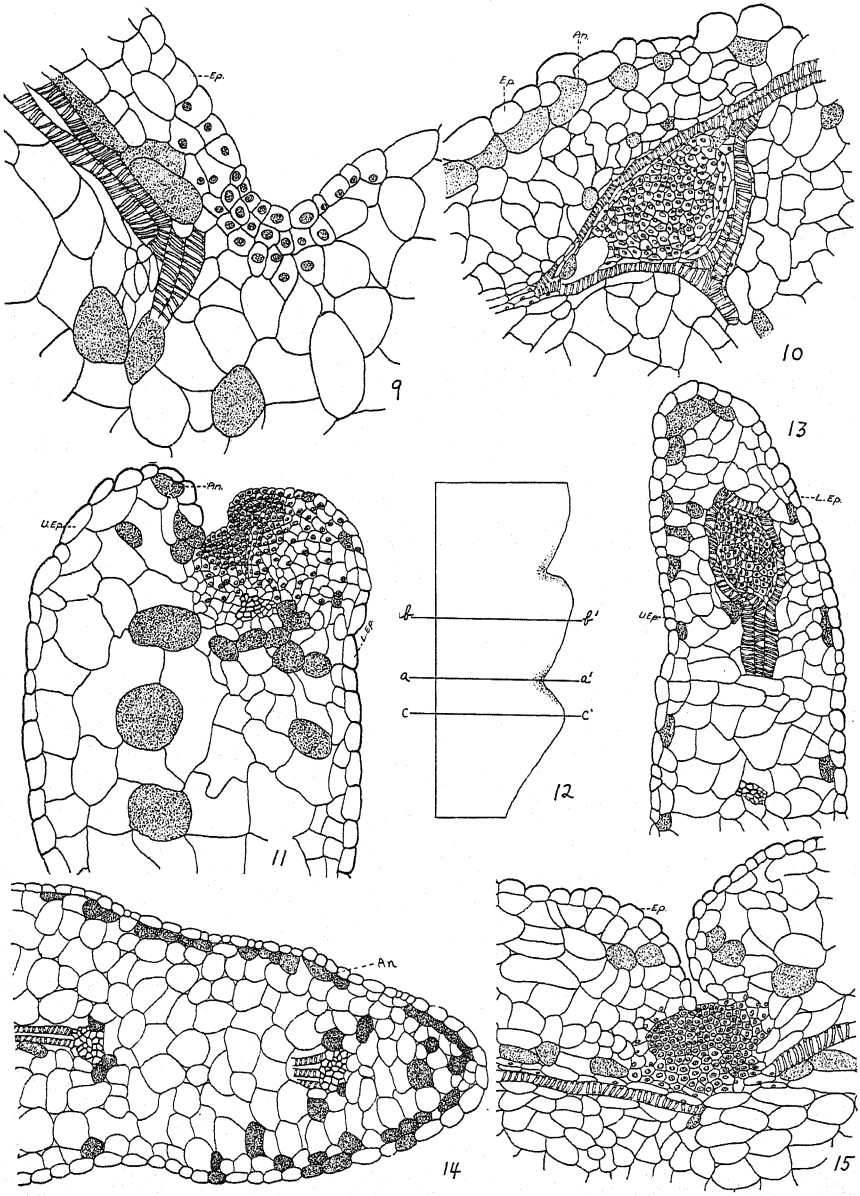
FIG. 1. Venation sketch of one of the primary leaves of a plantlet showing two notches and the two apex patches associated with them. Note the absence of apex patches in the region where crenations do not appear. × 12.

FIG. 2. Venation sketch of one of normal foliage leaves of plantlet showing many notches and relation of main veins and apex patches to them. × 9.

FIG. 3. Outline sketch of normal, adult foliage leaf showing form and crenations. × ½.



YARBROUGH: BRYOPHYLLUM



YARBROUGH: BRYOPHYLLUM

FIG. 4. Outline sketch of bracts commonly found in the inflorescences, that on the left being at the top and those to the right being found successively farther down the axis. Note the progressive elimination of crenations and disappearance of the petiolar portion in ascending the series. × ½.

FIG. 5. Sketch showing side view of growing point of stem of young plantlet. Primordia are 500 μ long and apex is 400 μ wide at level of growing point.

FIG. 6. Sketch of older leaf primordia. Note the basipetal development of crenations and wing development of the primordia. Each primordium is 2000 μ long and 750 μ wide.

FIG. 7. Cross section of mature leaf through developing embryo showing organization of first root primordium and resumption of cell division in the region of the shoot primordium. × 90.

FIG. 8. Sketch of leaves 3000 μ long and 1500 μ wide, showing further development of crenations and further growth of wing margins.

PLATE XXXVIII

FIG. 9. Tangential section through notch of young leaf about 2 mm. long showing appearance of meristem which builds up the foliar embryo. × 330.

FIG. 10. Tangential section of normal foliage leaf through the apex patch showing its relation to the vascular system and to the leaf margin. × 250.

FIG. 11. Cross section through normal foliage leaf taken at the level *a–a′* in figure 12. Note the organization of primordia in the foliar embryo of the notch. × 115.

FIG. 12. Outline sketch of small portion of normal foliage leaf showing two notches and giving levels of sections made in figures 11, 13, and 14. × 4.

FIG. 13. Cross section through normal foliage leaf (6 cm. long) taken at the level *c–c′* in figure 12 showing cross section view of apex patch. × 78.

FIG. 14. Cross section through normal foliage leaf (10 cm. long) taken at the level *b–b′* in figure 12 showing normal anatomy of leaf. × 78.

FIG. 15. Tangential section through notch upon leaf 3–4 mm. long showing large mass of meristem composing foliar embryo. Compare with figure 11 which is a cross section of foliar embryo at about the same stage of development. × 125.

NOTES ON THE GENUS *ANABAENOPSIS*

WM. RANDOLPH TAYLOR

(Received for publication December 16, 1931) [1]

INTRODUCTION

Two years ago there were sent to the writer for study, through the kindness of Dr. Eduardo Quisumbing of the Bureau of Science, Manila, P. I., a few samples of phytoplankton. While some of the material has not proven of notable character, two samples showed representatives of the relatively recently recognized genus *Anabaenopsis* (Wołoszyńska) V. Miller (7, 10), not attributed to the Philippines, and so worthy of special ttention.

The material for both samples came from Sampaloc Lake, in the town of San Pablo, Laguna Province, Luzon Island, in 1929. One sample, from the open lake, contained what appeared to be two similar species of short filaments; the other had scattered filaments of these species but was dominated by a third which formed helixes of considerable length. They are reported to appear as abundant autumnal waterbloom at intervals of 3–4 years, producing a very offensive odor.

Several species of *Anabaenopsis* have been described: *A. tanganyikae* (G. S. West) V. Miller and *A. circularis* (G. S. West) V. Miller from Africa (7, 9); *A. circularis* var. *javanica* (Wołos.) Elenkin and *A. Raciborskii* (Wołos.) Elenkin from Java (3, 10); *A. Elenkinii* V. Miller, *A. Arnoldii* Aptek., and *A. Arnoldii* var. *recta* Roll from Russia (1, 7, 8); and attributed to this genus also *A. hispanica* Gonz.-Guerr., *A. hispanica* var. *luteola* Gonz.-Guerr., and *A. Cuatrecasasii* Gonz.-Guerr. from Spain and Morocco (5, 6, 6a). The geographical range of the genus is seen to be wide. The general concept seems to call for a truly phytoplankton organism with rather rigid trichome, straight or curved increasingly to a helix of varying character; heterocysts occur at each end of the trichome and the relatively smooth spores are spherical or oval and generally remote from the heterocysts in position. The species *A. hispanica* and *A. Cuatrecasasii* depart somewhat from the sharp genus concept in that they have flexuous trichomes with spores, in one case spiny, adjacent to the heterocysts and apparently are not truly phytoplankton organisms. They are reminiscent of the plants which K. M. Drew (2) reported, she considering that hers were well known species of *Cylindrospermum* with heterocysts, and sometimes adjacent spores, at both ends of the trichomes. The Spanish plants do not urgently concern us, since they do not resemble the Philippine plants, so they will not be

[1] Papers from the Department of Botany, University of Michigan, No. 360.

considered further here. The remainder fall naturally into two groups: in one the trichomes are relatively short, straight or in 1–3 coils, and sharply contracted at the cell ends or moniliform, with short rounded heterocysts; this includes *A. circularis*, *A. Elenkinii* and *A. Arnoldii*. The others, *A. tanganyikae* and *A. Raciborskii*, have longer or more extensively coiled trichomes little or not constricted, and with oval or elongated heterocysts. Both of these groups are represented in Africa, in Java, and in the Philippines. The finding of three forms in Sampaloc Lake, and the resemblance of two of these to Tanganyika species, necessarily led to a consideration of the exact morphology of the original African types. Through the great kindness of Professor F. E. Fritsch portions of three original samples were secured from Dr. W. A. Cunnington for study by the writer. Material of *Anabaenopsis circularis* (*Anabaena flos-aquae* var. *circularis* G. S. West) was sufficiently abundant in two of these, and the writer was able to come to a definite opinion respecting that species. Less information was secured regarding *Anabaenopsis tanganyikae* (*Anabaena tanganyikae* G. S. West), as trichomes were excessively rare.

A close inspection of the figures given by West (9, Pl. 10, figs. 2*a–e*) of his *Anabaena flos-aquae* var. *circularis* may well awaken suspicion as to the specific unity of the material with which he was dealing. Figure 2*b* has markedly compressed-spherical cells of greater diameter than shown by the other figures, where the cells, except after division, are much longer. Then one notes that figures 2*a* and 2*c* have rounded heterocysts while those of 2*d* and 2*e* have oval ones. These are all features of diagnostic value in the Nostocaceae, and considerable variability within a species calls for careful explanation. West's description of his new variety allows for the variation in shape of the vegetative cells and for considerable variation in diameter of the heterocyst, but is so brief, and the figures are so few and so small, that only a careful re-examination of the original material could set at rest the doubts awakened by the Philippine associations of species and encouraged by the variations in the figures cited.

It appears that, of the three Anabaenopses in the Philippine material, one (resembling *A. tanganyikae*) is not to concern us immediately. The other two rather resembled, the one figures 2*a* and 2*c* of West, and the other figure 2*b*. As the writer had satisfied himself that these two Philippine species were distinct, and had prepared diagnoses of them with what appeared to be satisfactorily contrasted characters, the differences between West's figures impressed him sharply immediately he attempted to decide which of the Philippine plants should be identified with the Tanganyika species. It was obvious that, if different, both could not be identified with West's concept, and even if that with longer cells was called *A. circularis* and the other *A. circularis* var. *javanica*, no stable decision had been reached, because of the variation between West's figures, for the differences between them were greater than those between his figure 2*b* and the characters of the Javanese variety.

African Material

Selecting the Tanganyika material from station 160 (Chamaluki, 15 Nov. 1904) as most abundant, a very careful series of drawings was made of all types of trichomes referable to *Anabaenopsis*, in the formalin preservative accompanying them. At first trouble was anticipated in quickly distinguishing *Anabaena flos-aquae* and the variety *circularis*, *sensu* West's figure 2*b*, but it was soon found that the more compressed cells, paired young intercalary heterocysts, and differences in diameter sufficiently facilitated recognition. The preliminary drawings sorted out readily into three classes as suggested by the groups under West's figure 2, and suites of measurements and further drawings were made. The two other samples confirmed the observations made on that from station 160. As a result three plants may be defined:

Anabaenopsis circularis (G. S. West) V. Miller *emend.* (Pl. XXXIX, figs. 5–10).

Trichomes short, curved into a nearly complete circle or coiled into a short spiral of 1–1$\frac{1}{3}$ rarely (and usually just before fragmentation between two young heterocysts) 2 or 3 turns of 40–50 μ in diameter; trichomes 4.7–(*5.3*)[2]–6.5 μ in diameter, cells cylindrical or slightly barrel-shaped with moderately rounded ends, compressed at plane of contact, generally about 7.5 μ (1.4 diameters) long, but reaching 10.6 μ in average maximum length; color of contents indeterminable, texture slightly granular; heterocysts terminal at both ends of the trichome, slightly elongated, granule small but usually visible, diameter of heterocyst 5.6–(*6.5*)–7.5 μ, length 6.5–(*7.1*)–8.4 μ (ratio 1 : 1.09), pale straw-colored or colorless; spores not seen.

This is associated with West's original varietal name, first because it alone is in close conformity with the measurements for trichome and heterocysts which he specifies, and second because it was the most abundant of the three forms, in so far as the samples in the writer's hands were concerned. As it seems necessary to designate one of them as the type of *Anabaenopsis circularis* (*Anabaena flos-aquae* var. *circularis*) this is, for the reasons given, the one selected. It is unfortunate that his measurements alone can be used to guide in the selection, but his description of cell form and selection of figures was apparently carefully prepared to cover all types. West's figures 2*a* and 2*c* seem to correspond to this description. Upon comparison with *A. Elenkinii* we find few features which can be cited in distinction from the Russian plant. Miller (7) offers characters which give little in trichome diameter or length to distinguish between them, but the cell shapes differ. In the heterocysts we have a difference also, namely that those in *A. Elenkinii* are spherical and perhaps a bit smaller on the average than the slightly oval ones of *A. circularis*.

[2] Where measurements are given thus: 4.7–(*5.3*)–6.5: the middle italicized figure represents an average from several measurements, and ratios are calculated, when given, between the average length and breadth figures.

ANABAENOPSIS **Cunningtonii** n.sp. (Pl. XXXIX, figs. 1–4).

Trichomes short, 110–333 μ or probably somewhat longer, averaging about 165 μ, straight or occasionally curved; trichomes 2.2–(3.6)–3.8 μ in diameter, cells cylindrical or slightly barrel-shaped with moderately rounded ends compressed at plane of contact, generally about 11.0 μ (3–4 diameters) long, but reaching 16.4 μ in average maximum length before division; color of contents indeterminable, texture slightly granular; heterocysts terminal at both ends of the trichome, oval or slightly obovate, somewhat flattened at contact with the trichome and usually with visible granule, 4.7–(5.8)–6.6 μ in diameter, 6.6–(7.9)–10.3 μ long (ratio of 1 : 1.36); trichome segments quickly separating with sufficient maturation of the heterocysts, which are formed in pairs; spores not seen.

This species is distinguished from the preceding by its straight trichomes with longer cells and more oval heterocysts. Except for these features it would be much harder to distinguish from *A. circularis* than either from the following type. Only with misgiving has the writer assigned any curved filaments to this species; care should be taken in measuring heterocysts not to measure the rounder shrunken content rather than the longer firm but hyaline wall. West's figure 2*e* (and possibly 2*d*) corresponds to this description.

ANABAENOPSIS ARNOLDII Aptek., *fa.* (African form) (Pl. XXXIX, figs. 11–14).

Trichomes short, curved to 270° or a complete circle, or into a spiral of 1–1$\frac{1}{3}$, rarely to 3 turns of 50–65 μ in diameter; trichomes 7.1–(7.7)–8.5 μ in diameter, cells compressed spherical to short barrel-shaped before division, length generally somewhat shorter than the diameter but from incidence of cells soon to divide averaging 8.3 μ long; color indeterminable, texture obscurely but coarsely granular; heterocysts subspherical, with thin membrane and granule at base, 6.6–(9.8)–10.3 μ in diameter 8.5–(9.4)–10.3 μ long (ratio 1 : 0.96); trichome segments soon separating between the paired young heterocysts; spores not seen.

This is the most easily distinguished of the three species the writer believes were confused in the original *Anabaena flos-aquae* var. *circularis*, by reason of its large, nearly round cells and heterocysts. It is very close to *Anabaenopsis Arnoldii*, differing in having shorter coils of trichome and larger, spherical rather than short oval heterocysts at maturity. Neither of these is enough to justify specific, and probably not even varietal, separation, nor does the difference in diameter of the coils greatly signify. The variety *recta* of *A. Arnoldii* is somewhat smaller than the type, as well as straighter, and otherwise as well as in its straightness differs more from it than does the Tanganyika plant described above. The writer would associate West's figure 2*b* with this species.

Aptekarj (1) stresses the paired intercalary heterocysts seen in his plant as a feature to distinguish it from the other *Anabaenopsis* species; this is not a character that can be thus applied. It appears that, in *Anabaenopsis*, the heterocysts are formed in pairs by the unequal subdivision of

two vegetative cells, each cutting off a smaller portion on the adjoining ends. These two portions then enlarge and mature in contact into the characteristic heterocysts of the species. Ordinary division of vegetative cells occurs near the middle of the parent cell. Formation of heterocysts is well shown in *A. Cunningtonii* (Pl. XXXIX, figs. 1, 4), in *A. circularis* (Pl. XXXIX, figs. 6, 8, 10, 9, 5), in *A. Arnoldii* fa. (Pl. XXXIX, figs. 11, 12, 14), and the well known constrictive cell division in many of the figures. This is not a new observation (1).

If, now, the Javanese *A. circularis* var. *javanica* be compared with *A. Arnoldii*, it will be seen that in form of cells and in size it is more closely related to that species than to *A. circularis* in the restricted sense. In fact, if *A. Arnoldii* be maintained distinct from *A. circularis*, so must be the Javanese plant. On comparison of the measurements of these two (*A. Arnoldii* and the var. *javanica*), it will be seen that the vegetative cell characters will hardly serve to distinguish them, and only the heterocyst size, particularly in that the heterocysts are smaller or larger than the average trichome diameter, serve to separate them. Consequently, it seems more in conformity with the obvious morphological similarities to associate with *A. Arnoldii* the variety *javanica* (Wołos.) n. comb., which differs from the species in having slightly more slender trichomes, smaller heterocysts and larger spores.

Finally, referring to the character which West uses, of the single large "pseudovacuole" in the vegetative cells of his plants, the writer would report having seen these structures, and would doubt, from the inconstancy of their position, size, number and even presence, that they are sufficiently reliable to afford a good diagnostic character. Most frequently they are indeed single, but occur to 5 or more in a cell, and as now observed after over 25 years in formaldehyde, do not present the aspect of pseudovacuoles.

Philippine Material

Turning now to the Philippine material, supplied to the writer dried on glass slips, and studied in distilled water, the most striking of the forms from Sampaloc Lake is to be described as new under the name:

Anabaenopsis **philippinensis** n.sp. (Pl. XL, figs. 1–7).

Trichomes elongated, spirally coiled in few to generally several (8–12) turns, of 12–25 μ in diameter, or very long and becoming loosely to closely entangled, when some parts usually retain a spiral disposition; diameter of trichomes 1.9–(3.3)–3.8 μ, fairly uniform; cylindrical cells faintly or generally not constricted at the ends, the septa very indistinct, but length at least twice the diameter; contents very pale bluish-green, very slightly granular, without pseudovacuoles; heterocysts terminal at each end of the trichome, 2.6–(3.5)–3.8 μ in diameter, 7.5–(9.4)–13.2 μ long (ratio 1 : 2.7), rounded conical, tapering from close to the base towards the rounded apex, straight to more often asymmetrical or curved, the membrane very pale straw-colored, thin, hardly thickened even at the juncture with the trichome, and basal granule absent; spores not seen.

From the richness of the dried mount, this plant apparently was not as abundant as the other two species, and was found more along the shore than in the open water. Plate XL, figures 1–7, shows its characteristics adequately; the more involved tangles of the very long trichomes, or chains of trichomes, were not drawn, but transitions from regular coils (figs. 1, 4) toward less regular, loose loops (figs. 3, 5) are shown. Except semidiagrammatically in figure 1, no attempt was made to show the position of the septa, which were ill defined. The curved heterocysts show best in figures 2 and 7. This plant falls into the group with *A. tanganyikae* and *A. Raciborskii*. Its elongated heterocysts distinguish it at once from the former, and relate it to the Javanese *A. Raciborskii*. However, they greatly exceed those of the latter species (which are 2.0–2.5 μ in diameter and 5–6 μ long) since they reach a length of 13.2 μ and also are curved. The trichome, however, is but a little more slender, contrasting its 1.9–3.8 μ with the 4.0 μ of *A. Raciborskii*, which would be slight difference were it not associated with that between the highly coiled trichomes of *A. philippinensis* and the straight or sparingly coiled trichomes of the Javanese plant. In view of what we know of the two collections, it is clearly desirable to treat the Philippine plant as distinct from its more southern relative, and from the African species. Respecting the latter, however, it may be noted that in a single trichome seem by the writer, the heterocysts were 5.5 and 6.0 μ long, and 2.0 and 2.5 μ in diameter, somewhat more slender than West (9) cites; the trichome was 2.0–2.5 μ in diameter, and the cells seemingly much longer than described; but the walls were indistinct.

Out in the central portions of Sampaloc Lake the phytoplankton was quite different, though still of *Anabaenopsis*. At first the writer was puzzled to account for the variability of the trichomes, but he finally decided that two organisms were intermixed. Of these the first in abundance is recognized as:

ANABAENOPSIS ARNOLDII Aptek. *fa.* (Philippine form) (Pl. XL, figs. 16–22).

Trichomes short, rarely straight or arcuate, usually coiled in 1, rarely 2 flat or spiral turns of 30–65 μ diameter; the nearly moniliform trichomes 7.5–(*8.7*)–9.4 μ in diameter, cells truncate spherical to truncate oval, equal or a little shorter or longer than broad, to decidedly longer just before division; contents pale blue-green, distinctly granulate, with pseudovacuoles; heterocysts terminal at both ends of the trichome, truncate spherical to slightly truncate ovoid, 5.6–(*7.9*)–9.8 μ in diameter, 7.5–(*7.9*)–9.4 μ long (ratio 1 : 1), nearly colorless, the wall firm, moderately thickened especially on the distinctly flattened end of contact with the trichome, but no granule in evidence; spores intercalary, not seen close to the heterocyst, quickly isolated by fracture of the trichome on one or both sides of the spherical or generally oval spore, 8.1–12.0 μ in diameter, 12.0–16.0 μ long, wall smooth, rather thin, colorless or faintly straw-colored.

This *Anabaenopsis* was very abundant, and the most richly represented of the three, judging from the heavily strewn mount. Its relatives are

A. Elenkinii, *A. circularis* and *A. Arnoldii*. The shape of the cells associates it inescapably with the latter species. On comparison of the measurements, it will be found that the plants of the present material show trichomes which probably exceed the type of *A. Arnoldii* in average diameter, and on the whole seem to have somewhat longer cells; the var. *javanica* and the African representative show more slender trichomes, the former seeming somewhat less deeply constricted. Taking the heterocysts, we find that they are probably on the average larger than the type and, by definition, larger than var. *javanica*, but notably smaller than the African form. Taking the spores, we find that those of the Philippine plant are intermediate between the type and the var. *javanica*, but relatively more slender. Other and minor differences, such as apparent absence of basal heterocyst granules, could be noted, but the writer is inclined to consider all of the differences of quite secondary specific value, and the establishment of varieties premature. In Plate XL figures 16–22 represent the Philippine form. In figure 17 at the lower portion the trichome is probably in process of division, and about to form a pair of heterocysts. It appears that the daughter trichomes separate promptly in this material, but the unequal size of the heterocysts on the two ends of several of the trichomes (figs. 17, 20) probably is to be recognized as indicating unequal age. Spores are shown in figures 21, 22, and are generally isolated by breaking of the trichome from one or both sides. The writer does not consider that they are ordinarily formed terminally in this material.

The third species of *Anabaenopsis* in the Philippine samples was principally found mixed in relatively small proportion with the last, recognizably different as more slender and less granular in aspect, with relatively longer cells. It may be described:

ANABAENOPSIS **luzonensis** n.sp. (Pl. XL, figs. 8–15).

Trichomes short, occasionally straight or curved, generally coiled in one, rarely 2, flat or spiral turns of 28–45 μ diameter, but trichome segments frequently adhering by the terminal heterocysts to form helixes of a few irregular turns; trichomes 5.2–(*6.4*)–7.5 μ in diameter, cells barrel-shaped, distinctly narrowed from the center toward the ends to ¾–⅔ their full diameter, 5.6–10.5 μ or perhaps somewhat more in length; contents pale blue-green, slightly granular, pseudovacuoles not obvious; heterocysts terminal at both ends of the trichome, oval, little flattened at the point of contact with the trichome, and granule not evident, 3.7 to more commonly 5.1–(*6.1*)–7.5 μ in diameter, 4.5 to more commonly 6.0–(*8.3*)–9.6 μ long (ratio 1 : 1.35), pale straw-colored to colorless; spores intercalary, not seen close to the heterocysts, quickly isolated by fracture of the filament on one or both sides of the oval spore, 8.1–9.7 μ in diameter, 12.2–16.2 μ long, wall moderately thick, straw-colored, apparently smooth.

This plant falls into the group with *A. Arnoldii*, *A. circularis* and *A. Elenkinii*, as did the last. From *A. Arnoldii*, with subspherical cells, it is obviously quite distinct. The revised description of *A. circularis* comes closer, but in that species the cells are rounded-cylindrical and

narrower than the present, which has barrel-shaped cells 20 percent wider than those of *A. circularis*, and heterocysts considerably longer. On the whole, by far the closest resemblance is with *A. Elenkinii*, for the cell shape corresponds well, although *A. luzonensis* is larger; however, the heterocysts of the new plant are oval while those of *A. Elenkinii* are described as nearly spherical, and the spores are also relatively longer. With the marked heterocyst difference, and with considerable differences in vegetative cells and spores, and apparently in the color of masses of the plant, it seems best to describe the Philippine plant as a new species; but should other forms be found differing in various degrees, as have been found for *A. Arnoldii*, and connecting this plant with *A. Elenkinii*, it would then eventually be regarded as a geographical form of that plant.

Descriptive Key to the Genus Anabaenopsis

1. Heterocysts elongated 2
1. Heterocysts short oval, spherical or truncated 4
2. Heterocysts straight; trichomes straight or sparingly coiled 3
2. Heterocysts curved, tapered, 2.6–3.8 μ × 7.5–13.2 μ; trichomes 1.9–3.8 μ diam., in long coils or tangled ..... **A. philippinensis**
3. Trichomes 2.4–2.6 μ diam.; heterocysts 2.0–3.0 μ × 5.0–6.0 μ, elliptical ..... **A. tanganyikae**
3. Trichomes 2.5–4.0 μ diam.; heterocysts 2.0–2.5 μ × 5.0–7.0 μ, tapered **A. Raciborskii**
4. Cells of trichome at full growth much longer than broad 5
4. Cells of trichome subspherical or compressed, seldom longer than broad ..... **A. Arnoldii**, *incl.:*
   a. Heterocysts 5.8–9.2 μ × 8–10 μ; trichomes 6.5–9 μ; spores 10.4–11.5 μ × 11.5–14.5 μ ..... *Russian type*
   b. Heterocysts smaller; trichomes 5–8 μ; spores 12–14 μ × 16–18 μ ..... *Javanese form*
   c. Heterocysts slightly larger, 6.6–10.3 μ × 8.5–10.3 μ; trichomes 7.1–8.5 μ diam. ..... *African form*
   d. Heterocysts 5.6–9.8 μ × 7.5–9.4 μ; trichome 7.5–9.4 μ; spores 8–12 μ × 12–16 μ ..... *Philippine form*
5. Trichome generally straight, 2.2–3.8 μ diam., cells about 3–4 diam. long ..... **A. Cunningtonii**
5. Trichome generally strongly arcuate to spiral, cells shorter 6
6. Cells rounded-cylindrical, 4.7–6.5 μ diam.; heterocysts 5.6–7.5 μ × 6.5–8.4 μ ..... **A. circularis**
6. Cells barrel-shaped 7
7. Trichomes 4.6–5.7 μ diam.; heterocysts 4.6–6.7 μ diam., spherical; plant mass blackish-brown ..... **A. Elenkinii**
7. Trichomes 5.2–7.5 μ diam.; heterocysts 5.1–7.5 μ × 6.0–9.6 μ, oval.. **A. luzonensis**

Diagnoses of New Species

Anabaenopsis **Cunningtonii** n.sp. (Pl. XXXIX, figs. 1–4).

Trichomata brevia, 110–333 μ (plerumque circiter 165 μ) longa, stricta vel raro curvata, 2.2–3.8 μ diam. (plerumque 3.6 μ); cellulis cylindricis vel medio subdistensis, apice rotundatis compressione mutua modice planatis, plerumque ca. 11.1 μ longa, maximis ante divisionem 16.4 μ, intus colore nondescriptis, textura paululo granulatis; heterocystibus utroque binis

terminalibus ovoideis vel subovoideis, a cellulis adjacentibus modice planatis, plerumque granulo visibili praeditis, diam. 4.7–6.6 μ (saepe 5.8 μ), 6.6–10.3 μ (saepe 7.9 μ) longis, mature trichomata cito in segmenta separantibus; sporis ignotis.—Specimen typicum ex Lacu Tanganyika, Africa (W. A. Cunnington), in herb. auctor, et Univ. Mich.

ANABAENOPSIS **luzonensis** n.sp. (Pl. XL, figs. 8–15).

Trichomata 5.2–7.5 μ (plerumque 6.4 μ) diam., brevia, interdum stricta vel curvata, saepissime spiralia vel helicoidia, 1–2-voluta, volutis 28–45 μ diam., saepe inter se ex heterocystibus nondum separantibus helices compositas irregulariter paucivolutas formantia; cellulis media parte distensis, utroque ad septum versus terunciatim angustatis, 5.6–10.5 μ longis, intus pallide cyaneoviridibus, paululo granulatis, absque pseudovacuolis; heterocystibus utroque terminalibus ovoideis, ad cellulas adjacentes vix planatis haud granulatis, diam. 5.1–7.5 μ (rarissime 3.7, saepissime 6.1 μ), 6.0–9.6 μ longis (plerumque 8.3 μ), pallide stramineis vel decoloratis; sporis 8.1–9.7 μ diam., 12.2–16.2 μ longis, intercalaribus nec prope heterocystes obviis, cito trichomatis fractione liberatis, parietibus modice crassis, stramineis laevibus.—Specimen typicum ex Lacu Sampaloc, Provincia Laguna, Ins. Philippinensibus (E. Quisumbing).

ANABAENOPSIS **philippinensis** n.sp. (Pl. XL, figs. 1–7).

Trichomata subcylindrica, 1.9–3.8 μ diam. (plerumque 3.3 μ), elongata, spiraliter 8–12-voluta, volutis 12–25 μ diam., vel elongata plus minusve intricata pro parte spiralia; cellulis cylindricis apice vix vel haud quaquam constrictis ca. 6.6 μ longis sed septis indistinctis, intus pallide cyaneoviridibus, paululo granulatis, absque pseudovacuolis; heterocystibus utroque terminalibus diam. 2.6–3.8 μ (plerumque 3.5 μ), 7.5–13.2 μ (plerumque 9.4 μ) longis, rotundato-conicis fere ex basi ad apicem rotundatum angustatis, rectis vel curvatis, membrana pallide straminea tenui vix ad septum incrassata, absque granulo basellari; sporis nondum visis. Specimen typicum ex Lacu Sampaloc, Provincia Laguna, Ins. Philippinensibus (E. Quisumbing).

SUMMARY

An account is given of the results of a re-examination of a portion of the original plankton material from which G. S. West described certain Anabaenas upon which the genus *Anabaenopsis* has since been founded. One of his species (*A. tanganyikae*) was scantily represented and probably as he interpreted it. His other concept (*A. circularis*) is to be viewed as three plants, *Anabaenopsis circularis s.s.*, *A. Arnoldii* fa., and *A. Cunningtonii* n. sp., all of which are described. An account is then given of the examination of Philippine phytoplankton samples, in which three Anabaenopses were found. One of these is also a form of *A. Arnoldii*, to which species the geographically proximate Javanese *A. Arnoldii* var. *javanica* (Wołos.) is related. The other two are new species, *A. philippinensis* being related to *A. Raciborskii* and the other, *A. luzonensis* to *A. Elenkinii*. These are described, the interrelationships in the genus are discussed, and a descriptive key to the genus is given.

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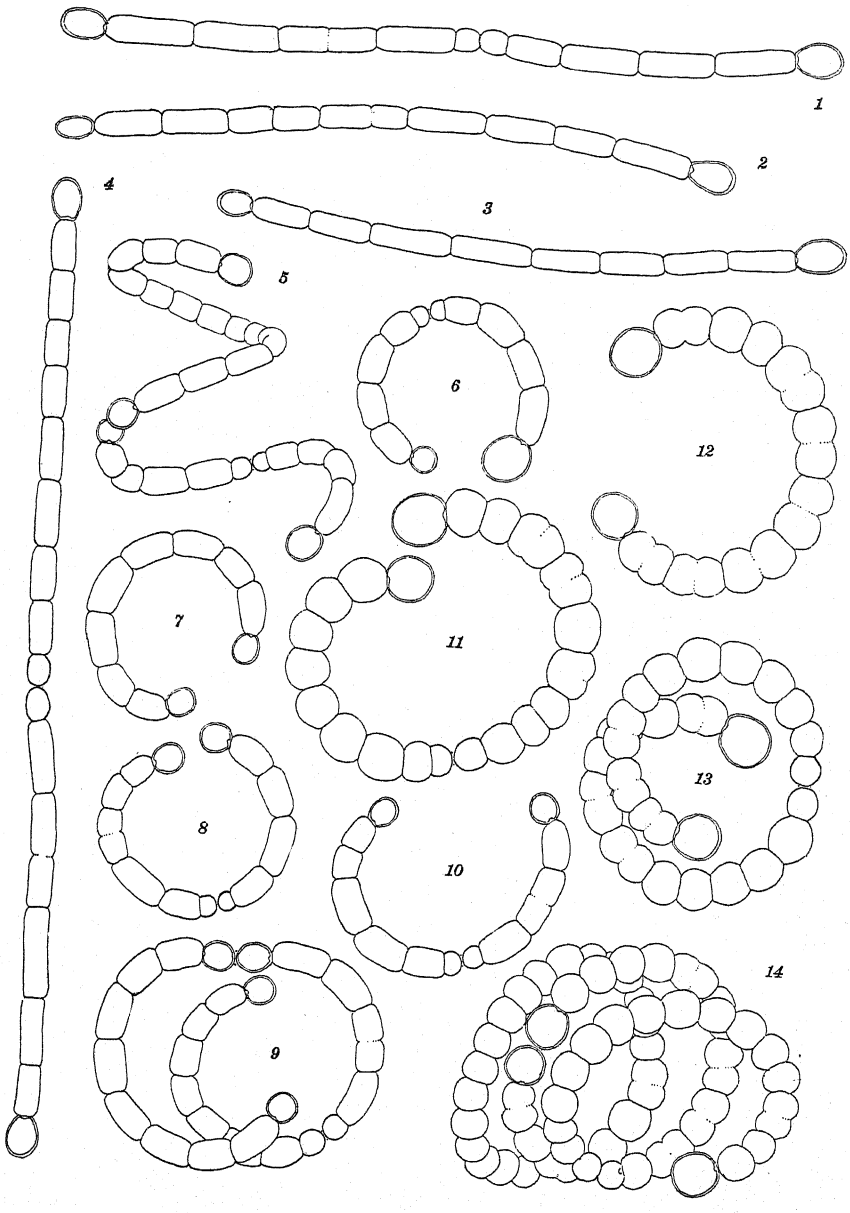

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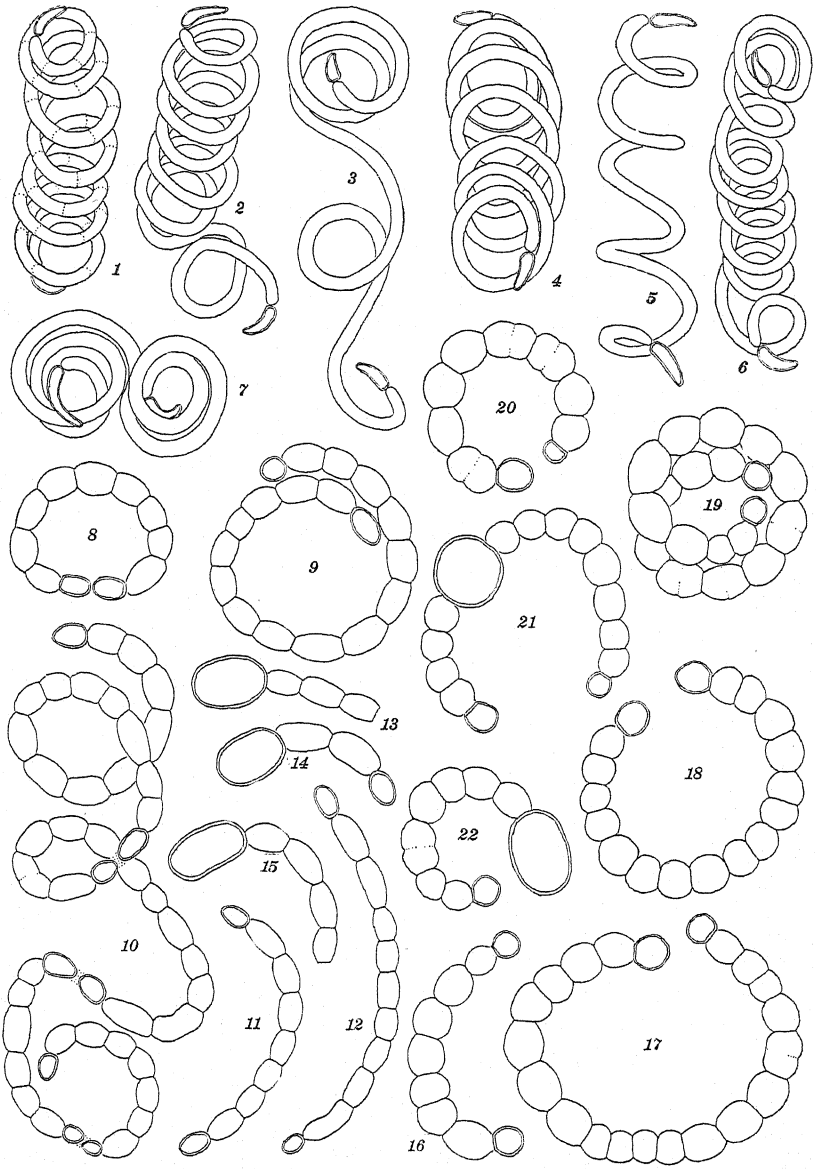

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DESCRIPTION OF PLATES

PLATE XXXIX

FIGS. 1–4. *Anabaenopsis Cunningtonii* (× 740). Trichomes showing heterocysts, and intercalary pairs of developing heterocysts.

FIGS. 5–10. *Anabaenopsis circularis* (× 740). Trichomes showing terminal heterocysts of various ages, and developing intercalary pairs.

FIGS. 11–14. *Anabaenopsis Arnoldii* (African form) (× 740). Trichomes showing intercalary and terminal heterocysts.

PLATE XL

FIGS. 1–7. *Anabaenopsis philippinensis* (× 606). Trichomes showing heterocysts; cell wall limits partly indicated in figure 1.

FIGS. 8–15. *Anabaenopsis luzonensis* (× 606). Trichomes showing heterocysts and (figs. 13–15) spores; figure 10 shows association in chain of trichomes.

FIGS. 16–22. *Anabaenopsis Arnoldii* (Philippine form) (× 606). Trichomes showing heterocysts and (figs. 21, 22) spores.

PROPAGATING SORGHUM BY CUTTINGS

H. E. Rea and R. E. Karper

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Although the sorghums are normally propagated by seed they may also be propagated from stem cuttings. Piedallu (1) reported in a French publication in 1919 that Minnesota Amber sorghum cuttings rooted readily. In 1930, Thomas and Venkatraman (2), working in India, stated that they were able to propagate sorghum vegetatively by planting it like sugar cane cuttings. Interest was first taken by the authors in propagating sorghums by means of stem cuttings during the fall of 1930 when the first trials were made with Sumac sorgo.

While field plantings of sorghum will always continue to utilize seed exclusively, the asexual propagation of sorghums should be useful to research workers and plant breeders. Cuttings should be highly desirable for increasing any lots of sorghum where only a limited number of seed are available or where it is desired to increase the lots without genetic alteration. Cuttings could often be used efficiently in increasing rare sorghum selections, importations, or hybrids.

The sorghum geneticist should find asexual propagation very useful in manipulating his plant material. While most of the operations feasible with cuttings can also be accomplished with seed, cuttings may supplement and facilitate these operations. In hybridization work cuttings may be used to maintain or increase plant material, whether it is heterozygous or homozygous. Individual sorghum plants, selected as parents in a given cross, may be maintained asexually after the cross has been completed and the seed matured. By this method the identical parent stock may be used for any number of subsequent crosses.

In artificial crossing of sorghum the number of hybrid seed matured is limited. For each seed planted a satisfactorily large population is usually produced. However, if for any reason additional F_2 seed were desired, the F_1 plants might be further propagated asexually and an extremely large population of F_2 seed might easily be produced.

Cuttings of sorghum may also be used for readily producing material for certain cytological studies. Within two weeks after placement many sorghum cuttings will produce an abundance of soft root tips excellent for cytological work. Likewise, cuttings offer an opportunity to produce pollen over an extended period, for within six to eight weeks many sorghum cuttings produce an abundance of pollen. Obviously other uses will suggest themselves as workers come to utilize sorghum cuttings.

Preliminary Experiments

In the first experiments in 1930, two-node stripped cuttings from second crop Sumac plants were placed in glass jar propagators with moist sand as medium. A total of 167 cuttings was made November 6 and 58.1 percent of them rooted successfully. The preliminary results obtained with Sumac sorgo encouraged trials with other sorghums. In the meantime field crops of sorghum had been destroyed by frost but fortunately there were a few plants of Sumac, feterita, and Johnson grass growing in the greenhouse. Additional trials were made with Sumac on November 20 and December 5, using 32 and 31 cuttings, respectively. Success was secured with 65.6 and 83.8 percent of these cuttings. On November 20 cuttings were obtained from feterita and handled in a similar manner to the Sumac cuttings. Out of a total of 44 cuttings, 30, or 68.2 percent, were successfully rooted. Johnson grass cuttings were made March 20, 1931, when 12 out of 50 cuttings, or 24 percent, were successful.

Material and Methods

In the spring of 1931 provision was made to have a variety of sorghums, representative of the different types or groups, available from which to make cuttings. Special plantings of each of several varieties of grass sorghums, sorgos, grain sorghums, broomcorn, and also Indian corn were made. The grass sorghums used were Tunis, Sudan, and Johnson. The sorgos were represented by Black Amber, Sumac, and Honey, while the grain sorghums included were Blackhul kafir, Hegari, feterita, Dwarf Yellow milo, and Standard Brown kaoliang. The broomcorn used was of the Acme variety and the Indian corn was Mosshart Yellow Dent. By July 17 most of these plantings had reached the stage of maturity desired for cuttings. The Sumac and Honey sorgos, however, were still immature but were used at that time since more mature plants of those varieties were not available.

From July 17–22, 1931, a total of 1075 cuttings was made from this material. The experiments set up were of three types: (1) Stripped two-node cuttings placed in glass jar propagators were used in the standard experiment. A total of 650 cuttings of this kind was made. (2) As one modification a smaller series of 100 two-node cuttings were placed in glass jar propagators without stripping the leaf sheaths from around the node. (3) An additional 325 cuttings, each consisting of many nodes, were made and placed in the open greenhouse bed. Moist sand was the medium used in all cases.

Making the Cuttings

The cutting material was collected from the field in separate lots of from ten to fifteen stalks of a single variety, prepared indoors, and none of the material exposed longer than necessary. Usually the cuttings were placed in the propagating medium in less than an hour after the plants were collected.

The stalks in the field were cut just above the crown. Three types of cuttings were made. Two groups were short and one was long. The short cuttings were from 9 to 12 centimeters in length and included a single internode and two nodes and were alike in the two groups except that the leaf sheath was removed or retained. The long cuttings were approximately 60 centimeters in length and included from 3 to 5 nodes. For convenience these three types were designated as two-node stripped, two-node unstripped, and many-node stripped cuttings.

In both groups of stripped cuttings the leaves were roughly stripped from the stalks. This usually left the nodes partially covered by remnants of the leaf sheaths. These remnants were carefully removed with the aid of a small pen knife. The stalks were then cut into the length desired. The lower cuts were made one centimeter below a node and the upper cuts five centimeters above a node. All cuts were angular and were made with a sharp steel blade. The two-node unstripped cuttings were cut directly without removing the leaves. Both groups of the stripped cuttings were inspected carefully to see that all nodes bore uninjured and completely exposed "eyes." In the case of all the sorghums these "eyes" were stem buds, but with corn they appeared to be ear buds. No inspection was possible for the cuttings where the leaf sheaths were not removed.

Placing and Care of Cuttings

All cuttings were placed in sharp, medium fine, sand, free of clay, with a moisture content of approximately 8 percent. The two-node cuttings were placed in glass jar propagators and the many-node cuttings in the open greenhouse bed. No attempt was made to sterilize the plant material; however, the sand and jars were fairly free of micro-organisms. Just before placing the cuttings their upper tips were dipped in melted paraffin. Care was taken to prevent covering the stem bud.

In placing the two-node cuttings in the glass jar propagators the jar was filled about one-fourth full of moist sand, cuttings placed in an upright position and pressed down in the sand. The jars were one-half gallon in size and conveniently accomodated five cuttings. After the cuttings were placed, moist sand was added to finish filling the jar to approximately three-fourths full, leaving about 2 centimeters of the tops of the cuttings uncovered. Finally the jars were loosely closed with screw tops without the use of rubber gaskets and moved to a permanent location. No water was added to the sand in the propagators and no special temperature was provided. The average outdoor temperature during the period was 83° F.

The long cuttings were heeled in moist sand in the greenhouse bed. About 5 centimeters of the tops were left uncovered and the bases of the cuttings were covered from 10 to 15 centimeters deep. The material placed in the greenhouse bed was watered daily. Within a few days after placing the long cuttings they began to buckle and tended to assume an

upright position. Because of this it was necessary to cover the butts deeper and to fill in with sand underneath the tops.

Records

All sorghum cuttings were left in the propagating medium for at least four weeks before they were taken up for examination. In the case of cuttings placed in glass jar propagators, their general condition and progress was observed from time to time and the experiment continued until roots began to show along the surface of the propagator. The records obtained at the end of the several experiments varied to some extent. A count of the total number of cuttings and the number successfully rooted was made in each experiment. For a cutting to be classified as successfully rooted it was required to develop both roots and leaves and be in a healthy condition.

Limited information was secured concerning the cuttings that showed some development but could not be classed as successful. These cuttings were divided into several classes, one group showing both root and leaf growth while the other showed only leaf growth. Usually these cuttings were severely damaged by micro-organisms. The sorghum cuttings were found to develop roots from two sources. The most common origin was from the base of the stem bud. However, roots were observed to develop from the root band encircling the node of the mother cutting. Those roots coming from the root band have been designated as brace roots and a count was made of the number of cuttings showing the development of these roots.

Results

Stripped Two-node Cuttings

In presenting the results secured with these two-node stripped cuttings, attention is first directed to the percentage of cuttings successfully rooted. As seen in table 1, on the average, only 6.6 percent of the cuttings made resulted in healthy, rooted cuttings with leaf development. Many varieties showed successful cuttings. Hegari, Sudan, and Tunis cuttings were among the most successful. Of all the cuttings tried from these varieties, 22.0, 16.0, and 12.0 percent, respectively, were clean, healthy cuttings with good root and stem growth. Kafir, milo, Black Amber, kaoliang, Johnson grass, and Sumac sorgo produced only a limited number of thrifty, well rooted cuttings while Honey, feterita, and Acme broomcorn produced none. Indian corn was not observed to make any stem growth and only infrequently did it produce roots. As a group, the grass sorghums were most successful.

From an examination of those cuttings which could not be regarded as successful a large number were found that made satisfactory root and stem growth but which had been severely injured by disease prior to taking the records. The percentages of cuttings belonging to this class are given under the "Damaged" column in table 1. Although these cuttings had no

TABLE 1. *Results of Two-node Stripped Cuttings of Sorghum Propagated in Glass Jars*

Kind of Sorghum	Percent Cuttings Making no Growth	Percent of Cuttings Making Some Growth			Percent of Cuttings Producing Brace Roots
		Roots and Stems		Stems Only	
		Damaged	Clean		
Tunis grass	18.0	64.0	12.0	6.0	8.0
Sudan grass	48.0	36.0	16.0	0.0	24.0
Johnson grass	96.0	2.0	2.0	0.0	0.0
Black amber	10.0	70.0	6.0	14.0	74.0
Sumac	72.0	24.0	2.0	2.0	14.0
Honey	33.3	66.7	0.0	0.0	62.2
Blackhul kafir	52.0	40.0	8.0	0.0	4.0
Hegari	22.0	34.0	22.0	22.0	2.0
Feterita	42.0	58.0	0.0	0.0	10.0
Dwarf Yel. milo	44.4	48.9	6.7	0.0	0.0
Std. B. kaoliang	58.0	36.0	4.0	2.0	8.0
Acme broomcorn	4.0	96.0	0.0	0.0	78.0
Average	41.6	48.0	6.6	3.8	23.7

prospect of producing new plants under the existing condition, they did indicate their ability to reproduce themselves under a favorable environment. From an inspection of the percentages recorded for this group, it may be seen that Acme broomcorn, Honey sorgo, and feterita produced many cuttings with root and leaf development. Many of the stem buds of these cuttings grew to be 10 centimeters long and from 1 to 2 centimeters in diameter. In general, the root development of the "Damaged" cuttings was almost as extensive as the clean cuttings and in some instances the roots remained in a healthy condition. The shoot appeared to suffer first from the attack of micro-organisms.

A few of the unsuccessful cuttings made some shoot growth without roots developing at all. The percentages of such cuttings are shown in table 1. Those cuttings recorded were in a thrifty condition at the time of collecting the data. Only 3.8 percent of all cuttings used produced stem growth exclusively, the cuttings of hegari and Black Amber manifesting this type of growth more frequently than did the cuttings of other varieties.

Observations of sorghum cuttings in the process of striking root indicate that the stem bud is the active growing unit. Upon stimulation this bud elongates and begins to unfold. Shortly afterwards leaves begin to appear and root buds form at the base of the stem bud. These roots are from the stem bud and are not a part of the mother cutting. The sorghum stalks have primordial root scars or root bands immediately above the nodes and frequently roots are developed from this source (text fig. 1*A*). The new plant did not appear to be dependent on the development of these root

primordia but their development undoubtedly added much to the growth of the new plant.

Under the column "Brace Roots," in table 1, the percentages of all cuttings developing roots from root bands are given. On the average, only 23.7 percent of the cuttings produced brace roots while 54.6 percent

TEXT FIG. 1. *A*, Acme broomcorn cuttings showing proliferation of stem buds and growth of shoots and roots. Roots were developed both from root buds and stem buds. *B*, Sumac cuttings showing development of roots from stem bud. *C*, shoot growth made from cuttings in greenhouse bench by Tunis grass, Sudan grass, and Johnson grass (left to right). *D*, shoot growth made by Honey sorgo, Standard feterita, Standard Brown kaoliang and Acme broomcorn cuttings (left to right). Three sections showing no shoot growth included Sumac, kafir, and Dwarf Yellow milo.

produced roots from the stem bud. Only rarely were cuttings found to produce brace roots without also developing roots directly from the stem bud. In such cases as were observed usually the top stem bud was the only one to develop and it was well out of the sand. Furthermore, the brace roots were usually slower to start growth and developed slower than did roots from the stem bud.

Unstripped Two-node Cuttings

In studying the cuttings of the 1930 trials that failed to make root growth their stem buds were frequently found to be partly covered by a portion of the leaf sheath. This suggested that the failure completely to remove the leaf sheath might either inhibit or delay rooting from the stem bud. To test this possibility a series of cuttings were made in which the leaf sheath was left in place.

For the purpose of this experiment, one variety of each of the sorghum groups was chosen. Sudan grass, Honey sorgo, feterita, and Acme broomcorn were the varieties used. Twenty-five cuttings of each were made. The results secured are given in table 2. In order to facilitate comparison,

TABLE 2. *Comparison of Unstripped and Stripped Two-node Cuttings Propagated in Glass Jars*

Kind of Sorghum	Treatment	Percent Cuttings Making no Growth	Percent of Cuttings Making Some Growth			Percent of Cuttings Producing Brace Roots
			Roots and Stems		Stems Only	
			Damaged	Clean		
Sudan grass.....	Unstripped	36.0	20.0	44.0	0.0	36.0
Sudan grass.....	Stripped	48.0	36.0	16.0	0.0	24.0
Honey.....	Unstripped	40.0	16.0	4.0	40.0	12.0
Honey.....	Stripped	33.3	66.7	0.0	0.0	62.2
Feterita.....	Unstripped	44.0	56.0	0.0	0.0	4.0
Feterita.....	Stripped	42.0	58.0	0.0	0.0	10.0
Acme.....	Unstripped	24.0	60.0	16.0	0.0	20.0
Acme.....	Stripped	4.0	96.0	0.0	0.0	78.0
Average.....	Unstripped	36.0	38.0	16.0	10.0	18.0
Average.....	Stripped	31.8	64.2	4.0	0.0	43.6

the results secured with the stripped cuttings of the four varieties concerned have been included in this table. It will be seen that 16.0 percent of the unstripped cuttings was successful, compared to 4.0 percent of those stripped. This comparison would indicate that leaving the leaf sheath on the cutting was more favorable under the condition of this trial. Contamination from micro-organisms was very general in the glass jars and apparently the presence of the leaf sheath offered some protection to the buds of the unstripped cuttings.

Although the roots of the unstripped cuttings grew through the leaf sheaths rather generally, the presence of the sheaths delayed root development in some instances. This appeared true particularly for the Honey sorgo cuttings. For this variety 40.0 percent of the cuttings produced only stem growth. Also, the average for all varieties indicates a delay in root growth. Only 18.0 percent of the unstripped cuttings developed brace roots, compared to 43.6 for the stripped cuttings. Fewer cuttings produced roots from the stem buds on the unstripped cuttings. The percentages were 54.0 and 68.2 for the unstripped and stripped, respectively.

Long or Many-node Cuttings

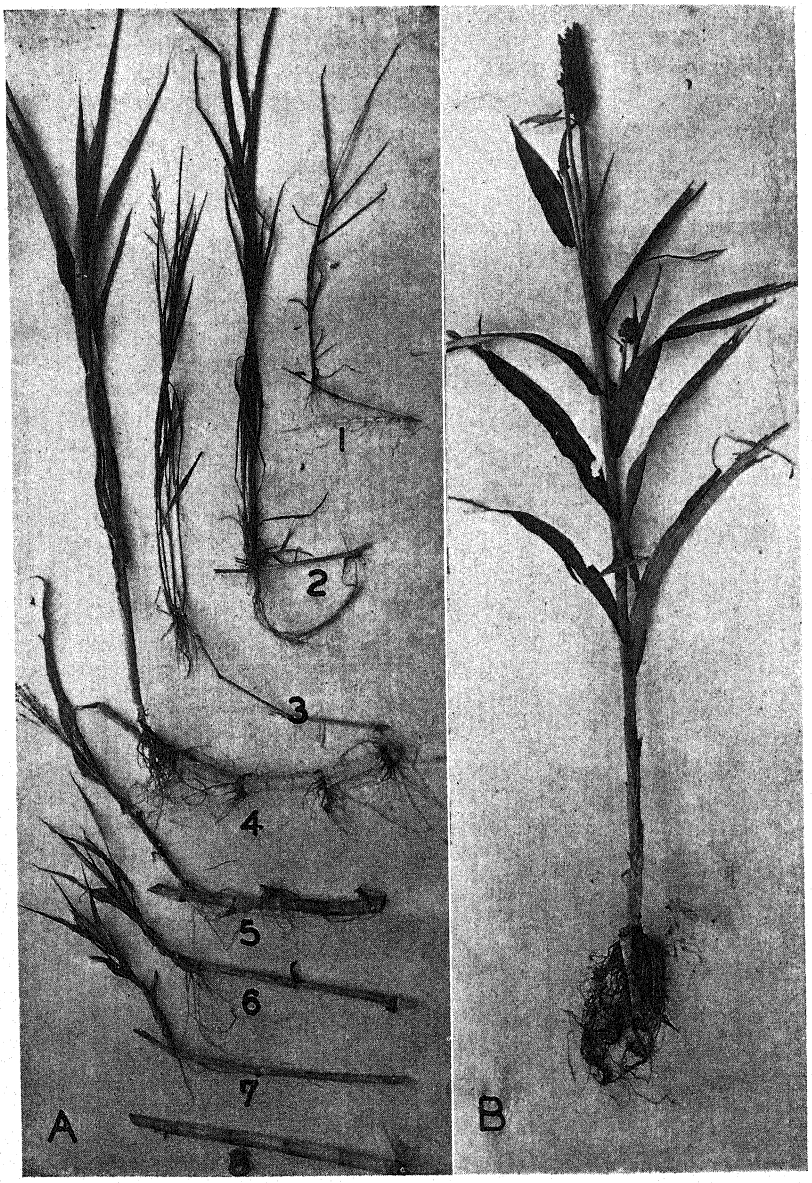
A total of 325 long, or many-node, cuttings was placed in moist sand in the greenhouse bench. The final records on these cuttings were not taken until September 23 (text fig. 2*A*).

The percentages of long cuttings that yielded one or more new and live plants are given in table 3. Broomcorn made a very high record since

TABLE 3. *Results of Many-node Sorghum Cuttings Placed in Greenhouse Bench*

Variety of Sorghum	Percentage			
	Cuttings Rooted and Stems Alive	Rooted but no Live Stems	Not Rooted	Joint Roots of Successful Cuttings
Tunis grass	52.0	0.0	48.0	88.5
Sudan grass	44.0	0.0	56.0	85.4
Johnson grass	32.0	0.0	68.0	84.4
Black amber	8.0	56.0	36.0	50.0
Sumac	16.0	48.0	36.0	58.3
Honey	60.0	24.0	16.0	100.0
Blackhul kafir	12.0	72.0	16.0	33.3
Hegari	12.0	64.0	24.0	44.4
Feterita	40.0	32.0	28.0	75.0
Dwarf Yel. milo	0.0	36.0	64.0	0.0
Std. B. kaoliang	24.0	12.0	64.0	38.9
Acme broomcorn	72.0	28.0	0.0	92.0
Average	31.0	31.0	38.0	62.5

72.0 percent of its cuttings was successful. Many of the broomcorn cuttings produced more than one new plant per cutting. In one instance the stem bud had put out seven side buds, although the shoot from the main bud had not yet reached the surface of the sand. All of these buds were healthy and vigorous looking. They were in active growing condition and each bud produced abundant root growth. Many of these bud clusters could have been sub-divided without injury. In general appearance they looked very much like a number of small bulblets. All the broomcorn cuttings counted as successful bore one or more shoots above the sand.



TEXT FIG. 2. *A*, shoot and root growth of representative cuttings propagated in greenhouse bench. 1, Johnson grass. 2, Tunis grass. 3, Sudan grass. 4, Honey sorgo. 5, Acme broomcorn. 6, Standard feterita. 7, Standard Brown kaoliang. 8, Mosshart Yellow Dent corn showing only root growth. *B*, mature Sumac sorgo plant grown from two-node cutting showing mother cutting attached.

However, all cuttings were given equal weight in calculating the percentage of success. The new broomcorn shoots grew to be only about 25 centimeters high but most of them produced mature seed before September 23.

Honey sorgo and Tunis grass cuttings also were quite successful. The percentages of new plants produced by these varieties were 60.0 and 52.0. The new stalks of both of these sorghums exceeded one meter in height by the time the final records were made and the growth was very vigorous (text fig. 1, *C* and *D*). The Tunis grass had matured seed and Sudan and Johnson grass were in full head. Sudan and feterita showed 44.0 and 40.0 percent, respectively, of successful cuttings. All of the sorghums produced some new plants except milo. On the average, 31.0 percent of the long sorghum cuttings was successful. This was somewhat higher than the average of 6.6 and 16.0 percent successes obtained from the two classes of shorter cuttings propagated in glass jars. No stem growth was found on the Indian corn cuttings although several of them produced good growth of brace roots.

In making the final observation on the many-node cuttings only infrequently was there more than one shoot per cutting in a healthy, vigorous condition except in broomcorn. Shoot growth was originally started at nearly every node but many of these were subsequently damaged by micro-organisms. The surviving shoots were generally from nodes only slightly covered by sand (text fig. 2*A*). Stem buds started shoot growth from the deeply covered nodes but only a few continued to grow. Root development on these deeply covered nodes, however, was very profuse. The origin of these roots was similar to those found on the shorter two-node cuttings used in other experiments except that brace roots were produced more frequently. The abundance of roots on these successful long cuttings is evident from the data presented in table 3. There were 15 cuttings of Honey sorgo that were successfully rooted and roots were found on 100.0 percent of the 45 joints available on these cuttings. Likewise, the percentage of available nodes producing roots on the successful cuttings of other varieties was very high. The percentages were, for broomcorn, 92.0; Tunis grass, 88.5; Sudan grass, 85.4; Johnson grass, 84.4; and feterita, 75.0. A similar condition prevailed for the other varieties.

Discussion

The adaptability of broomcorn to propagation by stem cuttings was distinctly greater than most of the other varieties of sorghums tested. Although the two-node stripped cuttings of broomcorn propagated in glass jars were all contaminated, these cuttings had previously made marked root and stem growth. Of a total of 50 cuttings used, 48, or 96.0 percent, had developed shoots and roots extensively. Unstripped broomcorn cuttings were fairly successful. The long, many-node broomcorn cuttings propagated in the greenhouse bench were very successful as all of these

cuttings produced roots and 72.0 percent of them grew into new plants. Of the 90 joints available on the successful broomcorn cuttings in the greenhouse bench, 92.0 percent of them rooted profusely. Suckering of the stem buds was also very general and this appeared to favor asexual reproduction.

The long, many-node cuttings of Honey sorgo resulted in a 60.0 percent success. Shorter Honey cuttings propagated in the glass jars were only fairly successful. Considering the extensive root development and vigorous stem growth of the cuttings in the greenhouse bench, however, better success was had in propagating this sorghum than with any of the others except broomcorn. Sumac sorgo yielded very few successfully rooted cuttings in the 1931 tests. This might have been due in part to the immature material used; however, the plants used for the Honey sorgo cuttings were still less mature. To say the least, a large proportion of Sumac cuttings will root under favorable conditions. In one trial during the fall of 1930 over 80.0 percent of the cuttings used were successful. On the other hand, with Black Amber sorgo the maximum percentage of success made in any of the trials was 8.0.

Many successful cuttings were obtained from the grass sorghums. The results secured in the open greenhouse bed were good for all three of the grass sorghums tested. Tunis grass produced new plants from 52.0 percent of the long cuttings used. These new plants were very vigorous from the start, grew rapidly, produced a number of off-shoots, and made very extensive root growth prior to taking the final records. Many of the new plants even matured seed. All of the cuttings propagated in the glass jars gave poor results but compared to the results of the other varieties the Tunis grass cuttings were among the best.

New Sudan grass plants were produced by 44.0 percent of both long cuttings and the unstripped short ones. The short, stripped Sudan cuttings were successful to the extent of 16.0 percent. The growth of the Sudan cuttings was almost as satisfactory as that of Tunis grass. With Johnson grass, a perennial sorghum, the cuttings placed in the glass jars were almost complete failures but 32.0 percent of the long cuttings in the greenhouse bench rooted and produced new plants. As a whole the grass sorghums were propagated more readily from cuttings than any of the other sorghums except broomcorn.

Although no uncontaminated new Feterita plants were obtained from the glass jar propagators, 58.0 percent of the cuttings of this variety did develop roots and stems before beginning to deteriorate. In the greenhouse bench experiment, 40.0 percent of the feterita cuttings produced thrifty, new plants. The maximum percentage of success for Dwarf Yellow milo was only 6.7 in glass jar propagators while in the trials with long cuttings on the greenhouse bench no new plants were produced. Kaoliang, kafir, and Hegari many-node cuttings produced a few new plants but the short cuttings in glass jars were badly damaged, and with the exception of Hegari,

only a few of the cuttings were successful. As a group the grain sorghums appeared to be less adapted to this type of propagation than any of the other sorghums.

Indian corn was the only crop plant other than the sorghums included in these propagating trials. Some of the corn cuttings produced root growth (text fig. 2, *A* 8) from the root bands above the nodes but no new plants resulted since no shoot growth developed.

These experiments indicate that sorghums may be readily propagated from cuttings. Conditions most favorable to successful handling of such cuttings are still to be worked out as soil organisms damaged a rather high percentage of the cuttings under the conditions of these trials. The fact that these experiments were conducted in the greenhouse under the very high temperatures of midsummer was probably responsible for a part of this damage. Had the rooted shoots been taken off of the mother cuttings, or the whole cutting been potted off or transplanted at the proper time, undoubtedly many of them would have developed into healthy, new plants. A number of the cuttings in the earlier trials were potted and later transplanted to the field where normal plants developed and produced seed (text fig. 2*B*). Also, a number of them developed and matured in the greenhouse. Healthier cuttings are maintained when the leaf sheaths are not removed but the shoots and their roots have some difficulty in breaking through the sheath. A larger number of new plants can be obtained by shallow layering of long, many-node cuttings in sand, removing and transplanting the shoots when well rooted, or letting the new plants grow in place. Single-node cuttings placed in the sand, pots, or directly in the field, should also be successful in propagating sorghum.

Summary

1. Stem cuttings were made of twelve types and varieties of sorghum and one variety of Indian corn. Results show that sorghums can readily be propagated from cuttings.

2. The types of stalk pieces used were stripped and unstripped two-node cuttings and stripped many-node cuttings. The two-node cuttings were propagated in glass jars and the many-node cuttings were heeled in the open greenhouse bed. The latter method proved the more successful.

3. Broomcorn appeared to be best adapted to propagation by cuttings. Honey sorgo and Tunis grass cuttings were also very successful. The other varieties showed considerable variation in the degree of their adaptability to this method of propagation. Cuttings of Dwarf Yellow milo were the least successful.

4. As a group the grass sorghum cuttings were generally successful and were exceeded only by broomcorn. The sorgo group showed considerable variation while the grain sorghums produced the lowest percentage of satisfactorily rooted cuttings.

5. Cuttings from Indian corn produced roots from the root band but did not develop any shoots.

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FLOWER VARIATIONS AND SYMMETRY PATTERNS IN *STELLARIA MEDIA*, AND THEIR UNDERLYING SIGNIFICANCE

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The literature that has accumulated on the morphology of the flower, especially with reference to the number of floral parts, in the genus *Stellaria*, is as abundant as it is perplexing; this is not difficult to understand, however, since the flowers, though never striking in appearance, are truly protean in their structure. Taxonomically the genus has presented considerable difficulty, as is attested by the large number of species and varieties that have been described, and by the different generic names that have been assigned. This confusion results in part at least from the variability within the flowers of the same variety, and even, in many cases, of the same plant.

Most outstanding in number and range of floral variations among the members of the genus is *Stellaria media*. The apparent fickleness of this species has been the cause of controversy, as will be shown more in detail below; and while much has already been written on this species, it is felt that a minute study of the flowers will clarify to a considerable extent, at least, some of the morphological and taxonomic questions that have been in dispute.

Showing as it does so many differences in the number and arrangement of floral parts, *S. media* is well adapted to the study of organization in the flower; clues to the underlying principles governing the relationships within the individual flower seem to lie in such a plant as this, which is apparently still rather plastic in many ways.

It is with these points in mind that the following data and interpretations of the flowers of *S. media* are presented. The results included here deal with a single variety of *S. media*,—a ten-stamened form, the sub-species *neglecta*, variety *typica*, of Béguinot (1910). Additional studies have been made on one of the other varieties, which will be published in the near future.

LITERATURE REVIEW

The literature of the genus *Stellaria* and especially of *S. media*, has been summarized from various angles. In view of the bibliographies published in recent years, by Béguinot (1907, 1910, 1920, 1921), Graebner (1916), and Matzke (1929, 1930), only that literature dealing with the particular aspects which are to be discussed below will be reviewed again. Béguinot

has described some thirty-two varieties of *S. media*, as well as four hybrids, and some related species. He based the varietal differences on such characters as the relative length of calyx and corolla (or the entire absence of the corolla), on the number of stamens, on the extent of the pubescence, and on the shape of the sepals. That such characters as the number of stamens vary is very evident, and is clearly brought out in Béguinot's work; and while there is even overlapping in the range of these stamen variations in different varieties, this character, as will be shown below, does offer a basis for the separation of certain forms at least. Whether all the varieties described by Béguinot will stand the test of further searching investigation remains to be seen; but that certain varieties do exist, seems to be unquestionable.

Kraft (1917) emphasized very strongly the influence of environment on certain parts of the flower in *S. media*, especially the perianth; poor nutrition, as well as very rich soil conditions, he showed, cause a reduction in the size and number of the petals, in the latter case perhaps through favoring vegetative activity at the expense of floral development, the size of the petals especially being affected. Kraft studied further the development of the flower, and presents ten floral diagrams, which show, to some extent, sepal-petal-stamen-carpel relationships, though no extensive study of these was attempted. Variation in the flowers of *S. media* has also been studied by Reinöhl (1903), who maintained that light, moisture, and the character of the soil as well as the age of the plants were important influences in determining the number of stamens; Burkill (1895) also studied this species and concluded that the age of the plants and temperature were controlling factors.

Among the older accounts of this plant should be mentioned first Eichler's (1875, 1878) book. Eichler's diagram has been rather generally adopted (Pax 1889, Rendle 1925), though as he himself pointed out it is an unusual case in the arrangement of the stamens. While Eichler's treatment is an excellent summary, his book is of course essentially a résumé of many families, and no one genus could be given very extensive consideration. As will be shown below, the position of the carpels of flowers examined in the present study differs slightly from that shown by Eichler; and while he mentioned the variation within the genus, he obviously could do little more. Eichler also discussed the general structure of the flower, especially from the standpoint of its origin. He considered and accepted, in general, Hofmeister's (1868) conclusion that the available space within the flower is an important factor in the arrangement of the floral parts, though he recognized also the "equilibrium" to be attained. While symmetry relations within the flower are well shown in many of Eichler's diagrams, he made no detailed study of *S. media* from this standpoint. Hofmeister's "law" that the available space plays a part in the determination of the position of floral parts on the axis was emphasized by

Sachs (1874) and Čelakovský (1875), though it was early realized that this could not be the only factor involved.

The general distribution of the stamens in *S. media* was briefly noted by Wydler (1851, 1859); his results, as far as they go, are in general agreement with the results given below; however, Wydler offered no detailed data and apparently made no extensive investigation of stamen variations.

The position of the carpels with reference to the sepals has been figured in two different ways. Wydler (1851) early studied and illustrated this species. The carpel orientation in his diagram is essentially the same as that shown later by Eichler (1878). Eichler's figure, in this respect, has been adopted in turn by Pax (1889), Engler and Gilg (1924), Rendle (1925) and Troll (1928), and is the same as that of Warming (1920). Le Maout and Decaisne (1868) gave a floral diagram of *Stellaria* which differs from the figures of Wydler and Eichler in the position of the carpels with reference to the sepals. Recently Pool (1929) has used Le Maout and Decaisne's figure.

The orientation of the parts of the flower is correlated, in a measure at least, with the arrangement of leaves on the stem. When the subject of phyllotaxy really first attracted attention is not definitely known; Thompson (1917) suggests that the Greeks and Egyptians may have noted some of its features. Within the last hundred years, however, the literature on this subject has grown to gigantic proportions; it has been summarized in some of its aspects in a previous publication by the author (1929).

According to the old interpretation, strongly emphasized by Goethe (1790) and still rather generally accepted, the flower is a specialized shoot and the sporophylls are metamorphosed leaves. The flower parts may then give some indication of the original leaf arrangement. This theory receives some vindication in the arrangement of the sepals, which show a two-fifths spiral. The other parts of the flower, however, are cyclic. Wydler (1859) numbered the petals and stamens, as well as the sepals, according to their "genetic succession," thus indicating, perhaps, the original sequence of these parts on the axis—a feature that has been obliterated, excepting for the calyx, by the shortening of the axis of the flower. The data presented below are interpreted in the light of such a possible original spiral arrangement. In the stem as well as in the flower of *Stellaria* the original arrangement of the organs has presumably been modified, the phyllotaxy being decussate.

The basic foliar nature of the carpels has recently been questioned by Grégoire (1931). He looks upon them as organs *sui generis*, and without homologue in the vegetative equipment of the plant.

For more general considerations of form in plants, we are indebted to Sachs (1893). This author, in an ingenious analysis of the factors determining size and structure, showed how, as a rule, increasing size and specific differentiation go hand in hand. The fundamental plan upon which plants

of large proportions are constructed differs essentially from that upon which more minute plants are built. But, aside from the general validity of his conclusions, he was not concerned with the form of the flower in particular. The question of form in the stem, and more especially in the vascular tissue in the stem, has recently been treated by Bower (1930). Emphasizing mechanical strength, provision for conduction, and ventilated storage space as requisites for the more effective of the larger stem types, Bower dwells upon the importance of the proportion of surface exposure of dead xylem tissue to living, and points out how this has been attained through medullation, sculpturing of the outer surface of the xylem mass, disintegration of portions of the xylem, and general vitalisation of the bulk of the xylem. It is from this point of view that Bower approaches the question of size and form in plants. Among the more recent general discussions of the morphology of the flower may be mentioned that of Engler (1926). This treatise deals with the general morphology of the flower, and includes a discussion of the occurrence of staminodia; the more specific problems relating to the genus *Stellaria*, especially reduction in the number of stamens and the positions of most frequent disappearance, are hardly considered. Harper (1916, 1926, 1929) has recognized functional hypertrophy and the relation of surface tension to cellular bipartition, etc., as influencing form determination in the lower organisms. In the higher plants Sinnott (1922, 1927, 1931), Sinnott and Durham (1929) and Sinnott and Hammond (1930) have studied form inheritance, especially in the fruit, and have been able to establish this on a genetic basis.

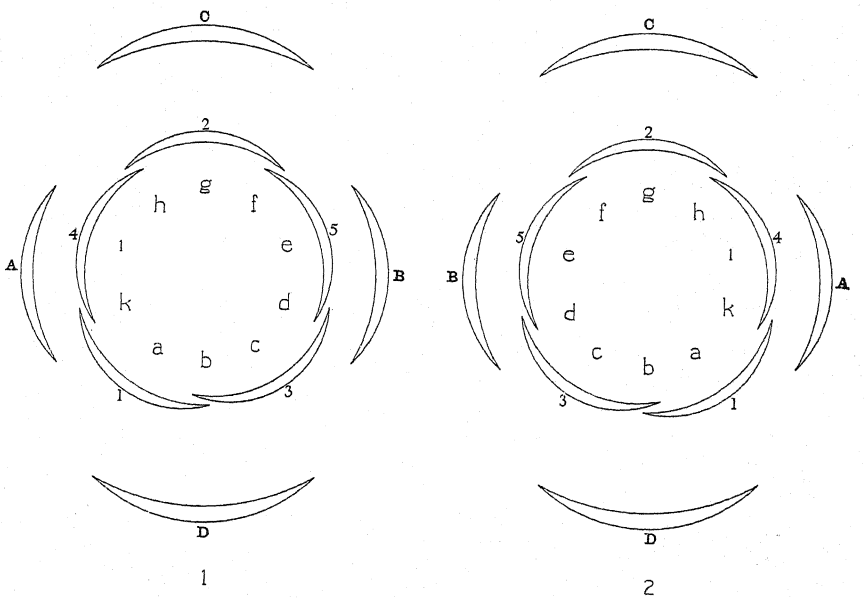
Data

The plants used were grown from seed collected at Rome, Italy. They were grown singly in pots and records kept of the variations in the flowers. The flowers were examined by means of a binocular dissecting microscope magnifying thirty-two diameters. All the plants were apparently of the same variety, though the flowers of certain ones showed some more or less constant differences from the flowers of others; all were apparently the *S. media neglecta* var. *typica* of Béguinot, which is accepted also by Graebner (1916). Béguinot, in his description of this variety, records the petals as slightly longer than the sepals; as he points out, however, this relation is not always maintained; the first flowers typically have the petals equal to or exceeding the calyx in length, while in the later ones they are usually shorter than the sepals. The later flowers of this, as well as of other varieties of *S. media*, are usually small and poorly developed.

The relationship between sepals and bracts has been described previously in detail for *S. aquatica*. As shown in figures 1 and 2 it is essentially the same for this variety of *S. media*. The only striking difference in the inflorescence is the more noticeable inequality between the development of the first branches borne at the same node in the young inflorescence;

this has already been described and figured in another variety of *S. media* (1930). Likewise the orientation of the sepals of the flowers, and of other floral parts as well, with reference to the leaves and branches of the stem, is as already described for *S. aquatica* and *S. media* (1929, 1930).

The flower typically has five sepals quincuncially arranged. The leaf arrangement of the stem is decussate, and this arrangement of the sepals is apparently an expression of the spiral phyllotaxy with two-fifths divergence. According to the older authors, especially Payer (1857), the sepals of *S. aquatica*, like those of other flowers with similar sepaline imbrication, indicate by the sequence and position of their origin, their original spiral arrangement. This spiral may form in either a clockwise or a counterclockwise direction (figs. 1 and 2, respectively). This difference is cor-



TEXT FIGS. 1 AND 2. FIG. 1. Arrangement of the sepals and bracts in a flower in which the sepals originate in clockwise sequence. FIG. 2. Arrangement of the sepals and bracts in a flower in which the sepals originate in counterclockwise sequence.

related with the method of branching, as has been described previously. Of 1701 flowers examined with this in mind, 866 had the sepals originating in clockwise sequence (as in text fig. 1) and 835 had them in counterclockwise sequence (as in text fig. 2). Data presented for *S. aquatica* show a slightly larger number of flowers with the sepals counterclockwise in origin. The calyx of these and similar flowers is usually regarded as consisting of only a single whorl; the five members quincuncially arranged really represent, however, two turns of a much flattened spiral. This is emphasized by the occurrence, only very rarely to be sure, of four-sepaled flowers having two

inner and two outer sepals (text figs. 111 and 112). Five such flowers were found. Of a total of 4652 flowers examined, all but seven had five sepals, and these all had four sepals. Of the remaining 4645 flowers, 4644—or all except one—had the usual quincuncial arrangement of the sepals. This flower is shown in figure 110. One flower had four sepals arranged as in figure 113. The sepals vary somewhat in size, both in the same and in different flowers, growing smaller, in general, with the diminishing size of the flowers as the season of blooming progresses. In the present study no striking variations in the form or character of the sepals of *S. media neglecta* var. *typica* have been found. This is in contrast to the not unusual occurrence of such variations, as already reported, in *S. aquatica*. The great constancy in the number of sepals per flower is also worthy of note, considerably less variation existing here than in the sepals of *S. aquatica*.

Typically there is a single two-lobed petal alternating with each sepal; the petals of the first flowers to bloom on a plant are more usually at least as long as or slightly longer than the sepals; those of the later flowers are shorter than the sepals. In *S. aquatica* there was some tendency for one of the petals to be missing. This tendency was not observed in *S. media neglecta typica*. Of the 4652 flowers examined only two had four petals, and these also had but four sepals, one petal alternating with each sepal (figs. 112 and 113). There is then apparently no notable tendency for the petals to disappear, at least not in plants grown in pots in the greenhouse. This would tend to support the contention of Béguinot that presence and absence of petals is associated with varietal differences, to an extent at least, in contrast to the view of Kraft that environment is the controlling factor. Even when the plants were old and bearing small flowers they retained the full complement of petals.

However, the occurrence of additional or supernumerary petals is by no means infrequent. In tables 1 and 2 are listed the ten positions lettered

TABLE 1. *Number of Additional Petals and Additional Petaloid Stamens, Number of Petals in Place of Stamens, and Number of Petaloid Stamens in Each Position in Flowers Having the Sepals Arranged in Clockwise Sequence as in Text Figure 1*

Sepal No.	1		4		2		5		3		Total
Position in flower	*a*	*k*	*i*	*h*	*g*	*f*	*e*	*d*	*c*	*b*	
Additional petal			1	3			1	1	15	2	23
Additional petaloid stamen									2		2
Petal apparently in place of stamen									16		16
Stamen petaloid		1	1		1				37	1	41

in the diagrams in figures 1 and 2, respectively; one petal is normally found in positions *b*, *d*, *f*, *h*, and *k*. In these tables, the additional petals found in each of the ten positions are enumerated. It is obvious that

supernumerary petals occur most frequently in position *c*; of a total of 53 extra petals, 36 occurred in this position. By additional or supernumerary petals are meant petals that are in excess of the normal five, and that are not formed in place of any other floral part.

TABLE 2. *Number of Additional Petals and Additional Petaloid Stamens, Number of Petals in Place of Stamens, and Number of Petaloid Stamens in Each Position in Flowers Having the Sepals Arranged in Counterclockwise Sequence as in Text Figure 2*

Sepal No.	1		3		5		2		4		Total
Position in flower	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*	
Additional petal		2	21		1		1	2	3		30
Additional petaloid stamen			5		1			1	1		8
Petal apparently in place of stamen	1		13								14
Stamen petaloid	1		35		1	1			1		39

The positions represented by the letters *a*–*k* in figures 1 and 2 are reduced almost to points in the actual flower; if the axis of the flower is considered to be elongated, as was originally the case if the flower is a modified shoot, then each of these positions would be extended into a line and the ten letters would then represent ten orthostiches. Figure 3 shows the parts of the flower, numbered to represent the "genetic succession" of Wydler and Schimper and Braun. Since sepals and petals alternate, the angle of divergence is smaller between the last sepal (5) and the first petal (1) than between two successive sepals or petals; a similar condition obtains between the last petal and the first stamen. If the supposed spiral of the petals is continued, an additional petal would occur in position *c*—just where it is actually most frequently found. An additional petal, then, occurs just where it would most naturally be expected on the assumption that the flower originally had the parts spirally arranged, that this spiral has been condensed, and that the parts were arranged as suggested by Schimper and Braun. According to their "genetic succession" the next petal should occur in position *c*.

As may be seen from tables 1 and 2, it happens not so very infrequently that a petal is present and the stamen which should occupy that position in the flower is missing. Of a total of 30 such cases, 29 occurred again in position *c*. Because of the closeness of the petaline and staminal whorls it is impossible to say with certainty that the petal occupies the place of the stamen. Preliminary investigation of the vascular system in a few such cases seems to indicate that the strand supplying such a petal originates like a petal strand, not like a normal stamen strand. The close relationship between stamens and petals in these flowers is indicated by the frequent occurrence of petaloid stamens. Such structures may be in addition to the full complement of floral parts, or one of the usual stamens may be more or less petaloid. Additional petaloid stamens were found in eleven

instances; as shown in tables 1 and 2, eight of these occurred in position *c*. Substitution of a more or less petaloid stamen for a normal stamen occurred 80 times among the 4652 flowers studied, and in 72 of these instances in position *c* again. Petaloidy of stamens may manifest itself in very varying degrees; sometimes the anther is just slightly whitish and dilated in appearance, giving only a slight suggestion of the petaloid character; from this minimum condition there are all intergradations, till the state is reached in which there is just a very small sac containing a few pollen grains on one lobe of an otherwise normal petal. Flowers found having one or more petaloid stamens had in each case the full complement of five normal petals —in other words no tendency was noted for any of the normal petals to become staminoid. Since all intergradations exist between stamens and petals, and since, as shown in tables 1 and 2, a petal may occur in position *c* while the stamen usually found there is missing, there may be a tendency to regard the petal as a transformed stamen, contrary to the old hypothesis of metamorphosis. An objection to this argument, however, is that the stamen and the petal may both be present.

The stamens of this variety of *S. media* show a much greater variation than any of the other parts, both in number and in the position that they occupy. The flower of maximum development typically has ten stamens, arranged in obdiplostemonous fashion, one corresponding to each of the positions lettered *a–k* in figures 1 and 2. The literature on the question of obdiplostemony has been summarized previously by the writer (1929) and the problem will be treated in a later investigation when the flower is considered from the standpoint of its vascular anatomy.

Floral diagrams showing the full complement of ten stamens are represented in figures 5 and 6, the only difference in these figures being in the arrangement of the sepals. As shown in table 3, this number of stamens was more commonly found in the variety under consideration than any other, occurring in 3597 flowers out of 4644 examined with the calyx normal. Of these 4644 flowers, some had one or more stamens modified, to a greater or less extent, into petals, and some also had an additional petal. This occurred for the most part in ten-stamened flowers. This maximum development may represent the original form in this genus, from which the fewer-stamened forms may have been derived. Such flowers in transverse section are as nearly bilaterally symmetrical as is possible with a calyx quincuncially arranged, the axis passing through positions *d* and *i* as indicated.

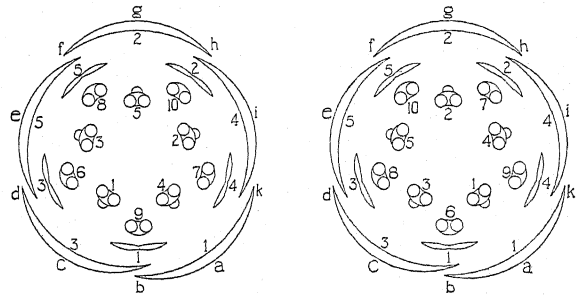
TABLE 3. *Numberof Flowers with 2–11 Stamens*

Number of stamens.....	11	10	9	8	7	6	5	4	3	2	Total
Number of flowers found.	9	3597	426	195	115	101	123	52	25	1	4644

There were in all 426 flowers with nine stamens. There are obviously

ten positions in which a stamen can be missing; these are represented by the letters *a–k* in figures 1 and 2. Table 4 shows the number of times that a stamen was missing in each of these ten positions in flowers having two to nine stamens; flowers having the sepals in clockwise sequence as in figure 1 and those having them in counterclockwise sequence as in figure 2 are here considered together, merely for convenience; as shown previously for *Stellaria aquatica* (1929) there is practically no other essential difference between flowers having the sepals in clockwise sequence and in counterclockwise sequence. Of 426 nine-stamened flowers examined, none was found with the missing stamen in position *e*, while 114 such flowers had the missing stamen in the adjacent position *f*.

As shown in table 5, the positions in nine-stamened flowers, in which a stamen may be missing, arranged in series, starting with the one of greatest frequency, are *f*, *a*, *g*, *d*, *k*, *b*, *h*, *c*, *i*, *e*. Although the total number of flowers with nine stamens was not large (426) the very great discrepancies in the frequency of the distribution of the missing stamen show without question that the arrangement of the stamens in such flowers is the expression of a definite internal organization. In *S. aquatica*, though the number of flowers with nine stamens was much smaller, this same position *f* was also most frequently involved. Figure 7 is a floral diagram of *S. media* with a stamen missing in position *f*. Positions *a* and *g* (figs. 8 and 9) also had stamens missing very frequently; positions *d*, *k*, *b*, *h*, *c*, and *i* (figs. 10, 11, 12, 13, 14, and 15, respectively) less frequently.



3 4

TEXT FIGS. 3 AND 4. FIG. 3. Flower parts numbered according to the "genetic succession" of the older authors, Schimper and Braun, Wydler, and Eichler. FIG. 4. Flower parts numbered according to a different "succession," suggested and discussed in the text.

If in the above sequence—*f*, *a*, *g*, *d*, *k*, *b*, *h*, *c*, *i*, *e*—the numbers of the stamens as determined by the "genetic succession" assigned by the older authors are substituted for the letters of the positions (as in fig. 3), the sequence will be 8, 4, 5, 6, 7, 9, 10, 1, 2, 3. It is evident that, according to this conception, the nine-stamened flower shows considerable order in the disappearance of the stamens.

However, if the positions *a–k* are arranged in descending series, depending upon the total frequency of missing stamens, not merely in nine-stamened flowers, but upon the total of missing stamens in all the flowers examined, the series is *f, d, k, h, b, a, g, c, i, e*, as shown at the bottom of table 5. If now the numbers of the "genetic succession" are substituted for the letters the sequence becomes 8, 6, 7, 10, 9, 4, 5, 1, 2, 3. This gives no evidence of regularity in the disappearance of the stamens. However, this "genetic succession," originated by Schimper and Braun and used by Wydler and others, was by no means universally accepted in the older literature (Eichler 1875). One obvious weakness of such a succession as indicating an original spiral is that in passing from one set of flower parts, for example the sepals, to another, the petals, which alternate with the first, it is impossible to keep the same angle of divergence. Thus, going the shorter way, from sepal 5 to petal 1 the angle of divergence is smaller than that between sepals 4 and 5. (Schimper and Braun went the longer way.) In passing from petal 5 to stamen 1 there is again a smaller angle, according to the accepted "genetic succession" (shown in fig. 3). But the stamens may be numbered in a different sequence, stamen number 1 being opposite sepal number 1, stamen number 2 opposite sepal number 2, etc. (fig. 4). This sequence is easily arrived at by increasing instead of decreasing the angle of divergence in passing from the fifth petal to the first stamen. Then the above mentioned series, denoting the frequency of missing stamens (*f, d, k, h, b, a, g, c, i, e*) becomes 10, 8, 9, 7, 6, 1, 2, 3, 4, 5. This would strongly indicate that the petaline stamens tended to disappear from the uppermost one down, the sepaline from the lowest one up. Considered in this way the sequence in the disappearance of the stamens takes on a new significance. In the pine cone the upper and lower sporophylls become sterile; here, according to this "succession," the stamens disappear from the top down and from the bottom up.

Considering further the broader aspects of floral variations in *S. media* as brought out in tables 4 and 5, before going in more detail into the organization of the particular flowers and their symmetry relationships, there are certain things that stand out. In the first place, a stamen is most frequently missing in position *f*. In four- and five-stamened flowers position *k* had a slightly greater number of missing stamens, but the differences there (one in each case) are so slight as to be negligible, while all the others and the total show stamens most frequently lost at *f*; as shown above this may be the uppermost one of an original spiral. Secondly, nine-stamened flowers not infrequently have a stamen missing opposite one of the outer sepals (positions *a* and *g*); in flowers with five and six stamens this was less frequently the case. In the third place, it may be said that in general the stamens opposite the petals are the first to disappear, and these in the sequence indicated in table 5; next stamens opposite the outer sepals are lost; those opposite the inner sepals are hardly ever missing. In the fourth

TABLE 4. *Position of Missing Stamens in Two- to Nine-stamened Flowers*

Sepal Number	1		3		5		2		4	
Position in Flower	*a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*k*
Position of missing stamen in 426 nine-stamened flowers	103	27	2	46	0	114	73	23	1	37
Position of missing stamens in 195 eight-stamened flowers	47	33	1	70	0	99	56	38	0	46
Position of missing stamens in 115 seven-stamened flowers	23	42	0	60	0	76	25	60	0	59
Position of missing stamens in 101 six-stamened flowers	20	61	0	75	0	88	16	71	1	72
Position of missing stamens in 123 five-stamened flowers	15	114	0	117	0	119	17	113	0	120
Position of missing stamens in 52 four-stamened flowers	33	48	0	49	0	51	28	50	1	52
Position of missing stamens in 25 three-stamened flowers	25	25	0	25	0	25	25	25	0	25
Position of missing stamens in 1 two-stamened flower	1	1	1	1	0	1	1	1	0	1
Total	267	351	4	443	0	573	241	381	3	412
Position of additional stamen in 9 eleven-stamened flowers	0	1	2	0	0	0	0	1	5	0

TABLE 5. *Positions in Table 4 Arranged According to Frequency of Missing Stamens*

	1	2	3	4	5	6	7	8	9	10
Positions in nine-stamened flowers arranged in order of frequency of missing stamens	*f*	*a*	*g*	*d*	*k*	*b*	*h*	*c*	*i*	*e*
Positions in eight-stamened flowers arranged in order of frequency of missing stamens	*f*	*d*	*g*	*a, k*		*h*	*b*	*c*	*e, i*	
Positions in seven-stamened flowers arranged in order of frequency of missing stamens	*f*	*d, h*		*k*	*b*	*g*	*a*	*c, e, i*		
Positions in six-stamened flowers arranged in order of frequency of missing stamens	*f*	*d*	*k*	*h*	*b*	*a*	*g*	*i*	*c, e*	
Positions in five-stamened flowers arranged in order of frequency of missing stamens	*k*	*f*	*d*	*b*	*h*	*g*	*a*	*c, e, i*		
Positions in four-stamened flowers arranged in order of frequency of missing stamens	*k*	*f*	*h*	*d*	*b*	*a*	*g*	*i*	*c, e*	
Positions in three-stamened flowers arranged in order of frequency of missing stamens	*a, b, d, f, g, h, k*							*c, e, i*		
Positions in two-stamened flowers arranged in order of frequency of missing stamens	*a, b, c, d, f, g, h, k*								*e, i*	
Positions of total arranged in order of frequency of missing stamens	*f*	*d*	*k*	*h*	*b*	*a*	*g*	*c*	*i*	*e*

place, of 4644 flowers with five sepals examined, every one had a stamen opposite the innermost sepal (position *e*)—a constancy that is remarkable; and only three flowers had no stamen in position *i*, and only four had none in position *c*, that is opposite the sepals next lower down. Such fixity of certain stamens, while others show such marked instability, is very striking.

The foregoing facts deal with the number of stamens in a flower, without taking into account their orientation with reference to the sepals. Thus in a ten-stamened flower the stamens are arranged in just one way, typically. However, from a purely mathematical standpoint, in a nine-stamened flower having the sepals as in figure 1, any one of the ten stamens could theoretically be missing; in other words a flower with nine stamens, mathematically considered, can have the stamens arranged in ten different ways, all having the sepals arranged as in figure 1. Eight-stamened flowers may have the stamens arranged in 45 different ways, etc. The number of combinations of eight stamens in a flower having ten positions may be derived from the formula

$$ {}_nC_r = \frac{n(n-1)(n-2)\cdots(n-r+1)}{r!} $$

in which n equals 10 and r equals 8 (or any other number of stamens—thus in seven-stamened flowers r would equal 7). Furthermore, since the sepals may be arranged either as in figure 1 or as in figure 2, it is obvious that there may be 2 different kinds of ten-stamened flowers, 20 different kinds of nine-stamened flowers, 90 different kinds of eight-stamened flowers. Since, however, flowers with the sepals as in figure 1 appear to be similar in structure to flowers with the sepals as in figure 2, being mirror images in floral diagrams, they may mathematically be considered together; this is done in the following table and floral diagrams. In table 6 are given the number of possible combinations of ten stamens, nine stamens, eight stamens, etc., and beside them the number that actually occurred.

TABLE 6. *Combinations of Stamens in Flowers Having 10 Stamens, 9 Stamens, 8 Stamens, etc.*

Kind of Flower	Number of Possible Combinations	Number of Combinations Actually Found	Percent of Possible Combinations Actually Found
10-stamened flowers	1	1	100
9-stamened flowers	10	9	90
8-stamened flowers	45	21	46.7
7-stamened flowers	120	29	24.2
6-stamened flowers	210	21	10
5-stamened flowers	252	14	5.6
4-stamened flowers	210	7	3.3
3-stamened flowers	120	1	0.8
2-stamened flowers	45	1	2.2
1-stamened flowers	10	0	0.0
0-stamened flowers	1	0	0.0
Total	1024	104	

As shown in table 6, the range of 0–10 stamens makes possible the existence of 1024 different kinds of flowers, each distinct in number or arrangement of stamens; and among 4644 normal five-sepaled flowers examined, just 104 were found; nor did these 104 occur with anything like equal frequency. It is evident immediately, then, that there is something more vital than mere "chance" which determines number and arrangement of stamens.

Figures 6–109 are floral diagrams of these 104 floral types having 2–10 stamens. These figures have the sepals either as in figure 1 or as in figure 2; actually, flowers exactly similar but having the sepals originating in the opposite way were found in nearly every case, and the two types of flowers are considered as one in each case as explained above. In table 7 the numbers of the floral diagrams together with the number of flowers of each type that was found are given. The number of flowers having the floral diagrams shown in figures 6–109 is given also by the number to the right of each number of the floral diagram. Thus the flower shown by figure 6 was found 3543 times; that shown by figure 7 was found 113 times, etc. The numbers given in table 7 include the flowers listed in tables 1 and 2 with one or more stamens partially or completely petaloid. This occurred for the most part in flowers with ten stamens. The numbers do not include flowers with supernumerary petals or petaloid stamens.

TABLE 7. *Frequency of Occurrence of Flowers Represented in Diagrams 6–109*

Diagram Number	Number of Flowers	Diagram Number	Number of Flowers	Diagram Number	Number of Flowers	Diagram Number	Number of Flowers	Diagram Number	Number of Flowers
6	3543	27	7	48	4	69	10	90	3
7	113	28	5	49	3	70	6	91	3
8	98	29	4	50	2	71	3	92	2
9	73	30	4	51	2	72	3	93	2
10	45	31	3	52	2	73	3	94	2
11	37	32	2	53	2	74	2	95	2
12	26	33	1	54	2	75	2	96	1
13	22	34	1	55	2	76	2	97	1
14	2	35	1	56	2	77	2	98	1
15	1	36	1	57	2	78	2	99	1
16	41	37	15	58	1	79	2	100	1
17	29	38	11	59	1	80	1	101	23
18	15	39	10	60	1	81	1	102	18
19	15	40	7	61	1	82	1	103	4
20	14	41	7	62	1	83	1	104	3
21	13	42	6	63	1	84	1	105	2
22	9	43	6	64	1	85	1	106	1
23	8	44	6	65	1	86	1	107	1
24	7	45	5	66	21	87	96	108	24
25	7	46	4	67	18	88	4	109	1
26	7	47	4	68	16	89	3		

The discrepancies between the numbers of flowers in tables 3 and 4 and those in table 7 (which are the same as those given on the floral diagrams) are due to the occurrence of flowers, with varying numbers of stamens, which have additional petals or additional petaloid stamens, or which

have two or four carpels. These are included in tables 3 and 4, but not in table 7. There were 51 flowers with additional petals (including two with two additional petals) and ten with additional petaloid stamens. Of these 61, one (fig. 82), is included in table 7; of the remaining 60 five had eleven stamens. There were eighteen flowers with four carpels, and there was one with two carpels. Of these nineteen flowers, one (fig. 110), did not have the sepals quincuncially arranged; one also had an additional petal, and one other had eleven stamens. Adding to the remaining sixteen flowers the 60 mentioned above, the four additional eleven-stamened flowers, the seven flowers with four sepals, the one with the sepals not quincuncially arranged, and the 4564 flowers in table 7, the total of 4652 flowers examined is obtained.

It is evident at once from table 7 that flowers represented by certain diagrams occur much more frequently than others. Thus in this variety of *S. media* the ten-stamened form with other parts of the flower normal occurred 3543 times out of a possible 4652. The later formed flowers on a plant often have fewer stamens than the first formed; but even in spite of that, ten-stamened flowers are by far the most frequent. As shown in figures 5 and 6, flowers with ten stamens are as nearly bilaterally symmetrical as is possible with a quincuncial calyx and as nearly radially symmetrical as possible with a quincuncial calyx and three carpels. Another very common floral type is that shown in figure 87; there the five stamens opposite the petals have been lost. A greater number of arrangements is possible in flowers with five stamens than in any other type occurring in *S. media neglecta*. In spite of this, 123 flowers showed only 14 different arrangements, and 96 of these 123 flowers had the stamens as in figure 87. Figure 87 again represents a flower that is bilaterally symmetrical and approaches radial symmetry. Twenty-four normal flowers with three stamens were found, and all of these had the stamens arranged as in figure 108—again a flower that is bilaterally symmetrical; although 120 different kinds of flowers are mathematically possible with three stamens, only this one occurred. Reduction in the number of stamens is very obviously going on in the flower of this variety of *S. media*—reduction from ten stamens to three, or possibly to two. The five-stamened flower represented in figure 87 is apparently a stage in this reduction; the three-stamened form shown in figure 108 represents the maximum reduction typically attained in this variety; and the symmetry of these three forms, among all the types of flowers that occur here, is striking. Other symmetrical flowers are represented in figures 10, 15, 17, 27, 28, 41, 42, 69, 100, 104, 106, and 113, and most of these occurred with some frequency. Symmetry relations, then, seem to play a part in determining the arrangement of the stamens in these various flowers. Grouping of the stamens on one side of the flower, as in figure 85, is not common.

While symmetry relations determine in part the flower pattern, other

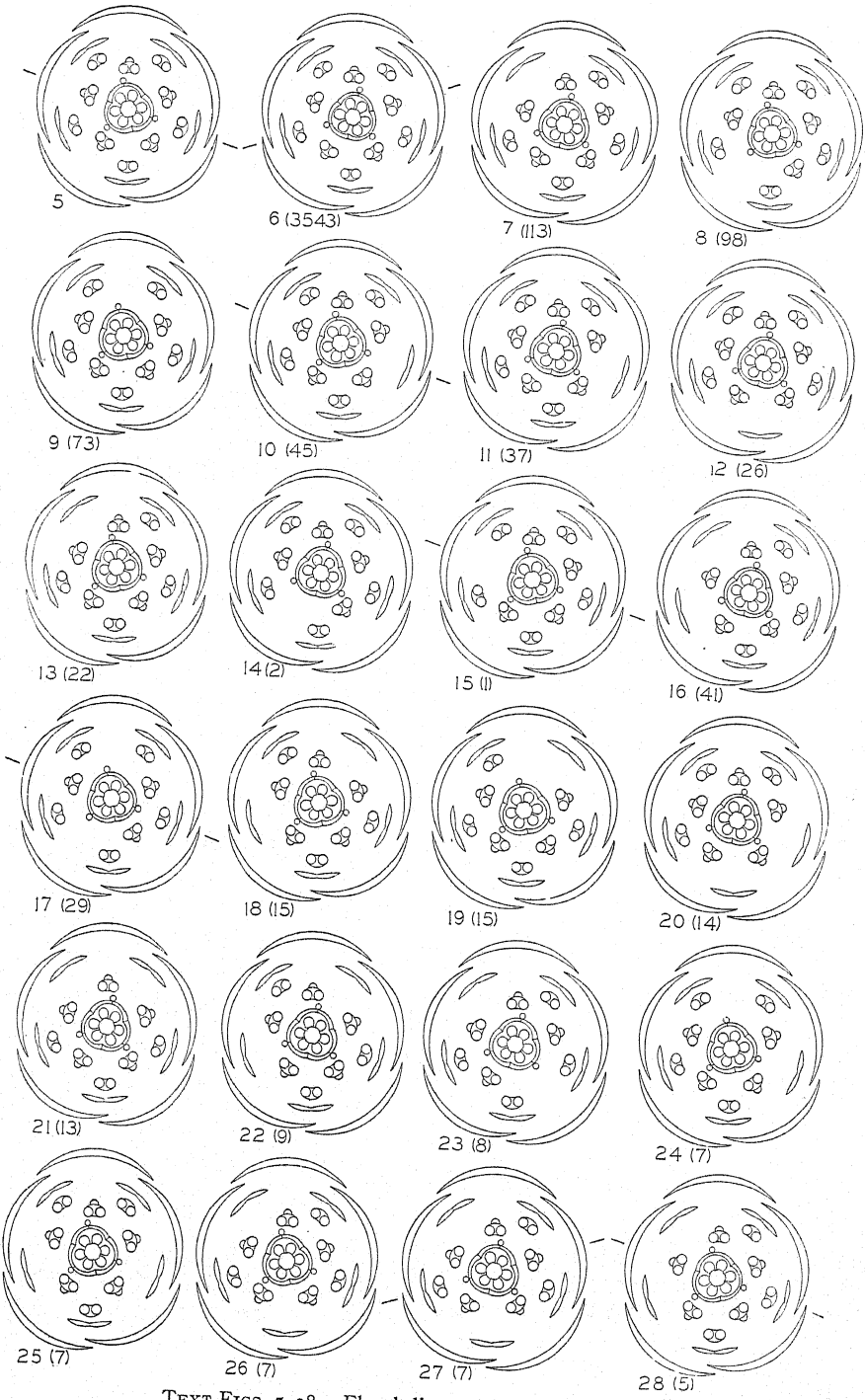
factors also influence the realization of this pattern. In a ten-stamened flower the stamens are symmetrically arranged and separated by equal distances; in a three-stamened flower the stamens are not separated by equal distances, but restricted to three of the original ten positions; hereditary limitations are apparently operative here. In spite of this, however, a bilateral symmetry is attained in the three-stamened flower. Symmetrical flowers, however, are not always the most frequent. Thus figure 15 represents a flower that is bilaterally symmetrical, but only one having this diagram was found (as shown in the number to the right of the diagram), while the flower represented in figure 7, which is not symmetrical, occurred 113 times.

As shown in table 6, the eight-stamened flower can mathematically have the stamens arranged in 45 different ways; 195 normal flowers with this number of stamens were found, arranged in 21 different ways; certain arrangements were much more frequent than others (figs. 16–36). Position *f* here again was most frequently without a stamen. As may be seen in those figures, in the majority of cases the missing stamens were either two of the petaline whorl (figs. 16, 18, 20, 21, 22, 25, 26, 27, 28, 30) or they were those opposite the two outer sepals (fig. 17). There is, even here, a foreshadowing of the typical five-stamened flower (fig. 87), which can be attained by the loss of three additional stamens of the petaline whorl, and of the typical three-stamened flower (fig. 108), arrived at by the loss of five petaline stamens in the flower represented in figure 17.

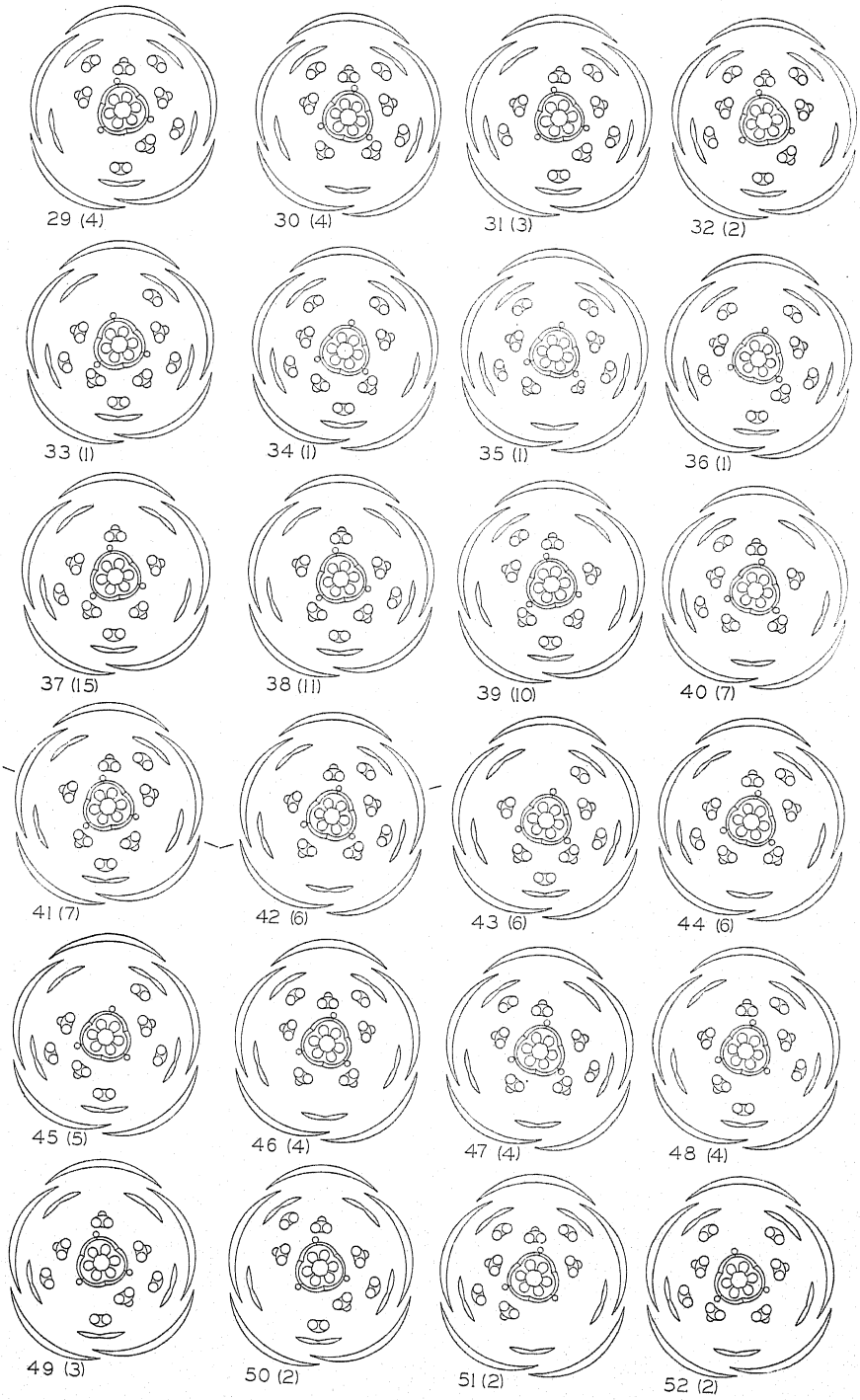
In seven-stamened flowers 120 different combinations are possible among the stamens; 29 of these occurred among the 115 flowers found with this number of stamens (figs. 37–65); 72 of these 115 flowers had three of the petaline stamens missing (figs. 37, 38, 39, 40, 41, 42, 44, 46, 47, 56) —suggesting again an approach to the typical five-stamened flower; of the remaining 43 seven-stamened flowers, eight (figs. 45, 55, 63) had the two stamens of the inner whorl, opposite the two outer sepals, missing—suggesting the three-stamened type (fig. 108).

Flowers with six stamens permit of 210 different arrangements in the ten positions; 21 of these occurred among 101 six-stamened flowers found (figs. 66–86). Of these 101 flowers, no less than 71 (figs. 66, 67, 68, 69, 70) had all four stamens missing in the petaline whorl—in other words, by the loss of the fifth petaline stamen they would have attained the typical five-stamened form (fig. 87); and eight of the remaining 30 six-stamened flowers had the two stamens opposite the two outer sepals missing (figs. 72, 73, 74) again indicating the condition attained in the typical three-stamened flower (fig. 108), which would be arrived at by the loss of three petaline stamens. These include the great majority of the flowers with six stamens.

In figure 87 is shown the typical five-stamened flower; and this may be looked upon as one of the important points in this line of reduction from the ten-stamened flower. It is obvious that this flower approaches a



TEXT FIGS. 5–28. Floral diagrams of *Stellaria media*.



TEXT FIGS. 29–52. Floral diagrams of *Stellaria media*.

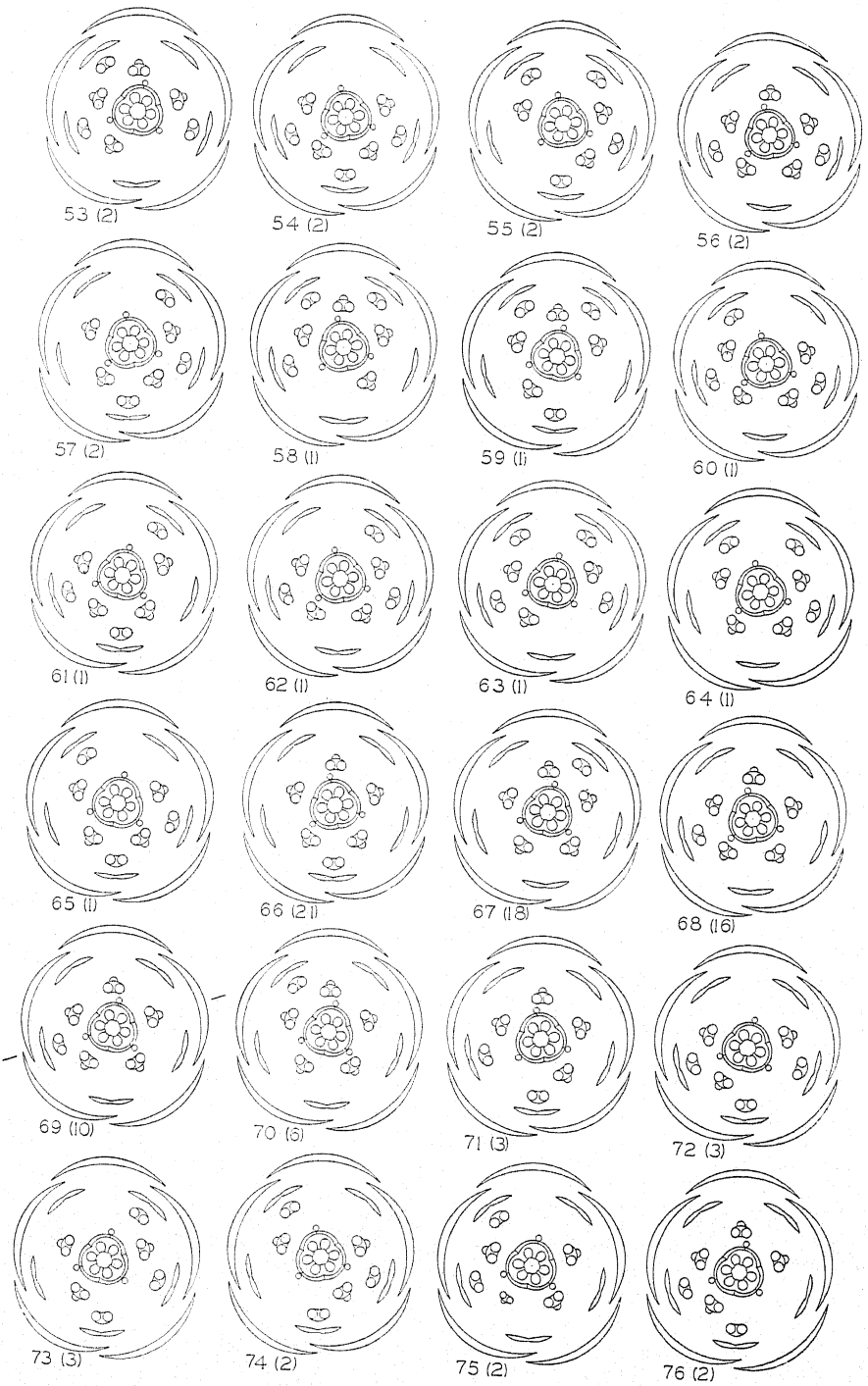
condition of stable equilibrium in the orientation of its parts. Although the five stamens can change their positions (mathematically) in 252 different ways, only 14 of these were found among 123 five-stamened flowers (figs. 87–100); and of these 123, there were 96 with the stamens as in figure 87. The symmetry of this flower again is immediately obvious—it being almost perfectly bilaterally symmetrical (as much so as possible with a quincuncial calyx) and as nearly radially symmetrical as the calyx and three carpels will allow. It is from this typical five-stamened form that other varieties of *S. media*, mostly smaller forms, carry the reduction to three and even fewer stamens. Although the flowers with five stamens were typically as in figure 87, six, nevertheless, had the two stamens opposite the two outer sepals missing (figs. 93, 97, 98, 99, 100).

Flowers having four stamens permit (mathematically) of 210 different stamen arrangements; among the 52 flowers found only seven arrangements occurred (figs. 101–107). As shown in figures 101 and 102, the four-stamened flowers most frequently have the five petaline stamens missing, and one of those opposite one of the two outer sepals—this being the case in 41 of the 52 flowers. Ten of the eleven remaining flowers (figs. 103, 104, 105, 107) have but to lose a single petaline stamen to attain the typical three-stamened form.

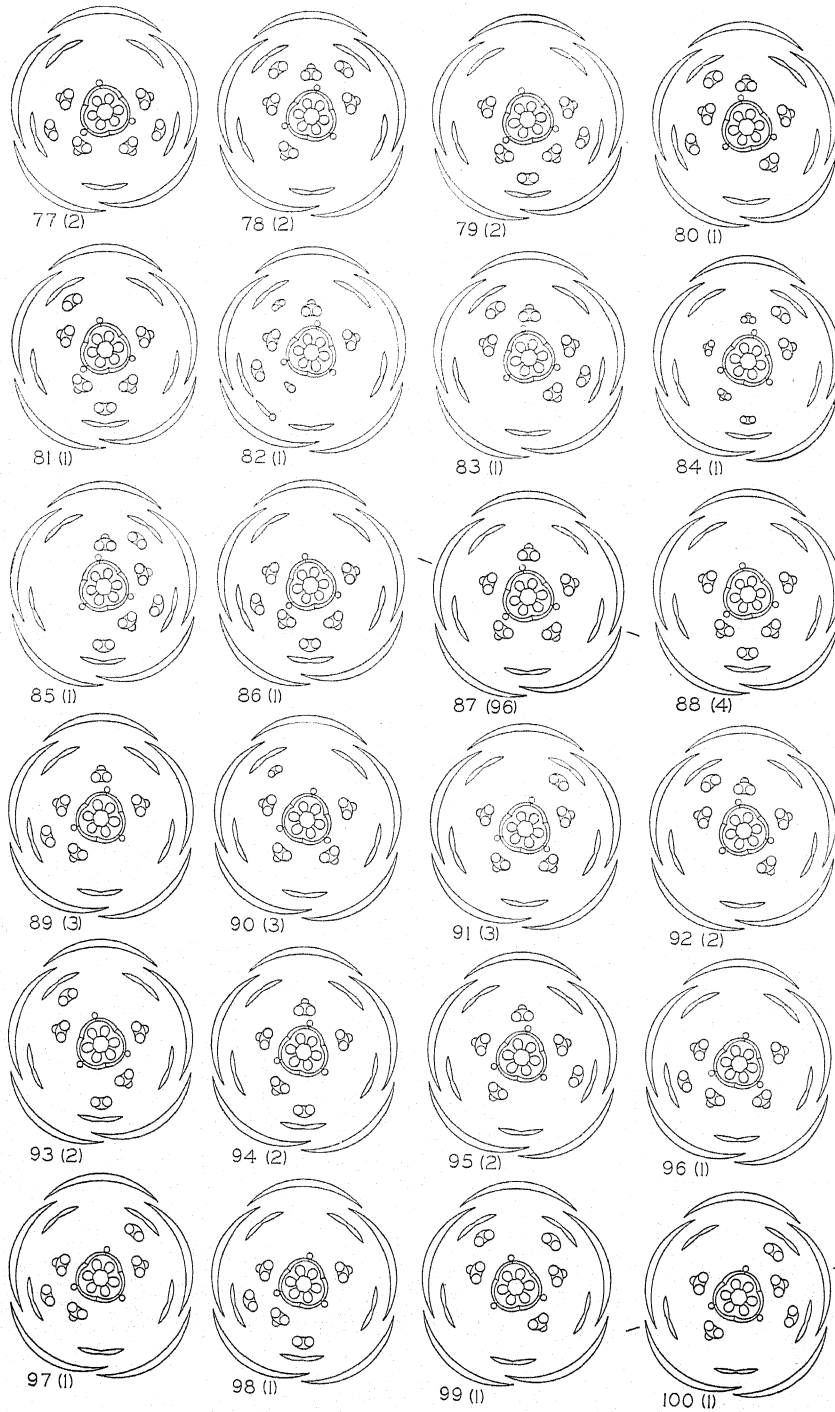
This three-stamened flower (fig. 108) seems to be a relatively stable thing. Although 120 different arrangements of these three stamens are mathematically possible, the twenty-four normal flowers found showed just a single one—that illustrated in figure 108. Other varieties of *S. media* also show the three-stamened form, some much more abundantly than *S. media neglecta* var. *typica*. The bilateral symmetry of this flower is again very noticeable. The principle of least surfaces, rather than the principle of functional hypertrophy, seems to be of importance here. The ten-stamened flower, with parts essentially in alternating whorls of fives, does not require such delicacy of response to contact and pressure as those flowers with fewer stamens and therefore with the parts not regularly alternating. Only a single flower with two stamens was found in this variety (fig. 109).

In this reduction of the stamens there are then three types that are outstanding—the flower with ten stamens which serves as the starting point (figs. 5 and 6), the flower with three stamens, which in this variety represents the ordinary maximum reduction (fig. 108), and the intermediate type with five stamens (fig. 87). Furthermore, flowers with eight, seven, six, and four stamens show a decided suggestion in the arrangement of their stamens of the typical flowers with only five and three stamens. However, this is only a decided tendency and holds by no means without exception.

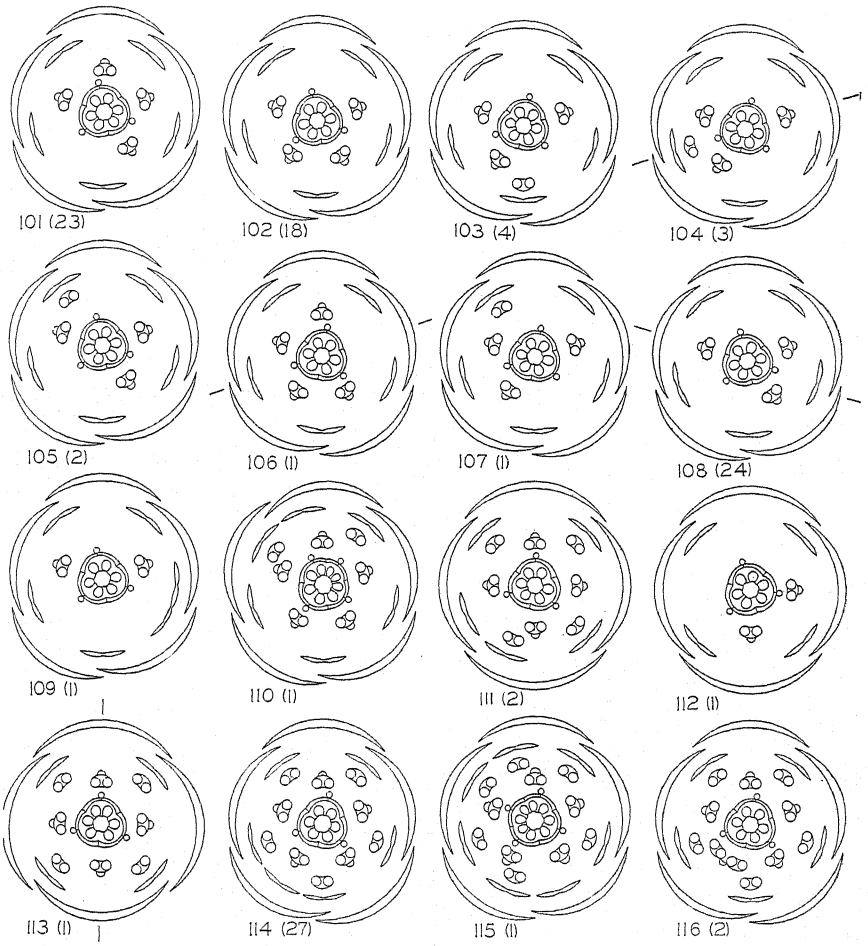
Three-stamened flowers (fig. 108) have stamens 1, 2, and 3 of the genetic succession of the older authors (fig. 3) and stamens 3, 4, and 5 of



TEXT FIGS. 53–76. Floral diagrams of *Stellaria media*.



TEXT FIGS. 77–100. Floral diagrams of *Stellaria media*.



TEXT FIGS. 101–116. Floral diagrams of *Stellaria media;* the numbers in parentheses indicate how frequently a flower with each diagram occurred.

the scheme shown in figure 4. The typical five-stamened flower (fig. 87) has stamens 1, 2, 3, 4, and 5 of both schemes.

The first flowers to bloom on a plant usually have ten stamens, the later ones frequently showing reduction. This is very evident when records of the flowers on individual plants are kept. Table 8 shows the number of flowers with three to ten stamens on one plant, kept in the greenhouse, from the beginning of its flowering on April 14 to June 31. Observations were made at intervals of one or several days; since a flower is usually open just a single day, some were missed; but a representative number of all the flowers was studied, and all the flowers open on any of the days listed in the table were examined. This table shows that the first

formed flowers usually have ten stamens; that those later formed may have ten or fewer; and, furthermore, that flowers with three, four, five, six, seven, eight, nine, and ten stamens all occur on the same plant. Records kept of other plants showed essentially these same characteristics.

TABLE 8. *Number of Flowers on One Plant with 10 to 3 Stamens on Respective Days*

Date	Number of Stamens 10	9	8	7	6	5	4	3
April 14..	1	—	—	—	—	—	—	—
" 15..	1	—	—	—	—	—	—	—
" 19..	2	—	—	—	—	—	—	—
" 21..	—	1	—	—	—	—	—	—
" 22..	3	—	—	—	—	—	—	—
" 23..	2	—	—	—	—	—	—	—
" 25..	3	—	—	—	—	—	—	—
" 29..	11	1	1	—	—	—	—	—
May 1..	10	—	—	—	—	—	—	—
" 2..	9	3	1	—	—	—	—	—
" 7..	1	3	—	—	—	—	—	—
" 8..	1	3	—	—	—	—	—	—
" 9..	3	—	—	—	—	—	—	—
" 12..	3	1	—	—	—	—	—	—
" 13..	3	1	1	—	—	—	—	—
" 15..	2	—	—	—	—	—	—	—
" 16..	2	—	—	—	—	—	—	—
" 17..	2	—	—	—	—	—	—	—
" 19..	6	—	—	—	—	—	—	—
" 20..	3	—	—	—	—	—	—	—
" 22..	2	—	—	—	—	—	—	—

Date	Number of Stamens 10	9	8	7	6	5	4	3
May 26..	6	3	2	1	—	—	—	—
" 28..	4	2	—	—	—	—	—	—
" 29..	2	3	1	1	—	—	—	—
" 31..	—	3	1	—	1	—	—	—
June 4..	2	2	1	—	1	2	1	3
" 5..	3	2	—	1	—	1	—	—
" 6..	2	1	—	1	—	—	—	1
" 7..	1	—	—	—	—	—	—	—
" 9..	1	—	—	—	—	—	—	—
" 10..	3	1	—	—	—	—	—	—
" 11..	3	2	—	—	—	—	—	1
" 12..	—	—	—	—	1	1	—	—
" 13..	2	2	1	1	2	—	—	—
" 16..	—	—	1	1	—	1	—	—
" 18..	1	—	—	—	—	1	1	—
" 20..	—	—	—	1	—	—	1	—
" 21..	1	—	1	—	—	—	—	—
" 23..	—	1	—	1	—	1	—	—
" 25..	—	—	—	—	—	1	—	—
" 31..	1	—	—	—	—	—	—	—

S. media neglecta var. *typica* normally has a single pistil composed of three carpels, with three stigmas; the capsule usually splits into six valves. The floral diagrams given by Wydler (1851), Eichler (1878), Pax (1889), Warming (1920), Engler and Gilg (1924), Rendle (1925) and Troll (1928) all have one carpel directly opposite to sepal 2 (figs. 1 and 2), and the two other carpels oriented symmetrically on the axis thus established. Le Maout and Decaisne (1868) also figured a three-carpeled *Stellaria* with the carpels oriented somewhat differently; their diagram has been adopted by Pool (1929). Kraft (1917) remarks that the position of the carpels in relation to the sepals is not absolutely fixed, and figures it in flowers with varying numbers of floral parts. The figure of Wydler and Eichler has the carpels oriented in the way most usually found in tricarpellary flowers having five sepals quincuncially arranged. Eichler (1875, 1878) gave diagrams of flowers in various families in which this arrangement occurs. However, this is by no means universal; the same author also diagrammed flowers, having five sepals similarly arranged and three carpels, in which the carpels are not oriented in this way with reference to the sepals. In a few cases Eichler's diagrams of flowers with a five-parted calyx and three carpels show the same orientation that is given in figures 5–109: *Delphinium Staphisagria*, *Tropaeolum majus* and *Aesculus Hippocastanum*. It is significant that many years ago Rohrbach (1868, 1869) described and

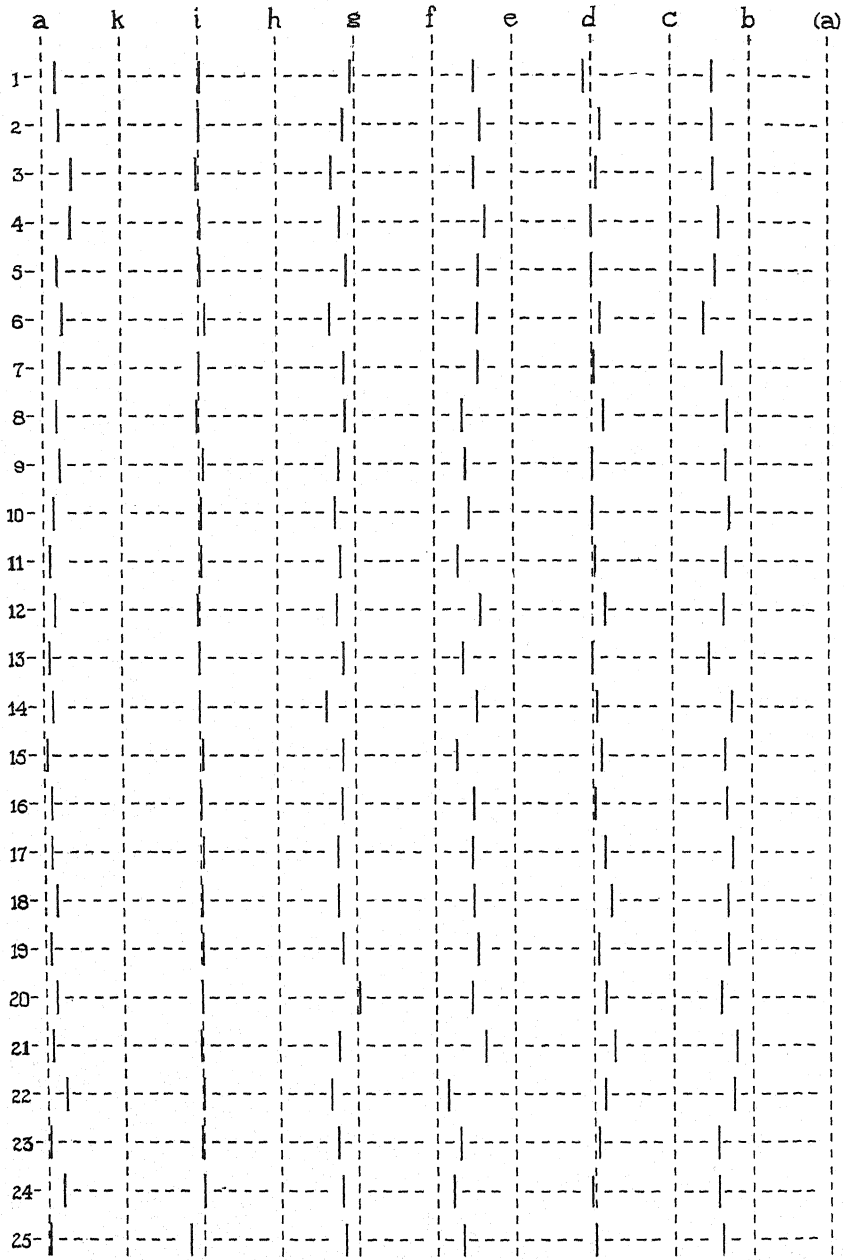
figured the orientation of the three carpels in *Silene* and *Tropaeolum* exactly as they are given in figures 5–109; he stated that this was their more usual, though not unalterable, position. For the Caryophyllaceae, at least, this orientation has not received general acceptance.

To determine the position of the three carpels by the position of the styles is difficult and unsatisfactory in *S. media* because of the ease with which they bend, and because of their delicacy; and to use the swellings on the ovary, while somewhat more accurate, also leaves much to be desired. The capsule splits into six valves; three of the lines of dehiscence are opposite to, three alternating with, the three stigmas (Eichler 1878, Pax 1889). The splitting of the capsule, then, seems to offer the best method of determining the position of the carpels. When the capsules are mature they are closely enveloped by the sepals, thus making the rather accurate determination of the position of the carpels with reference to the sepals possible.

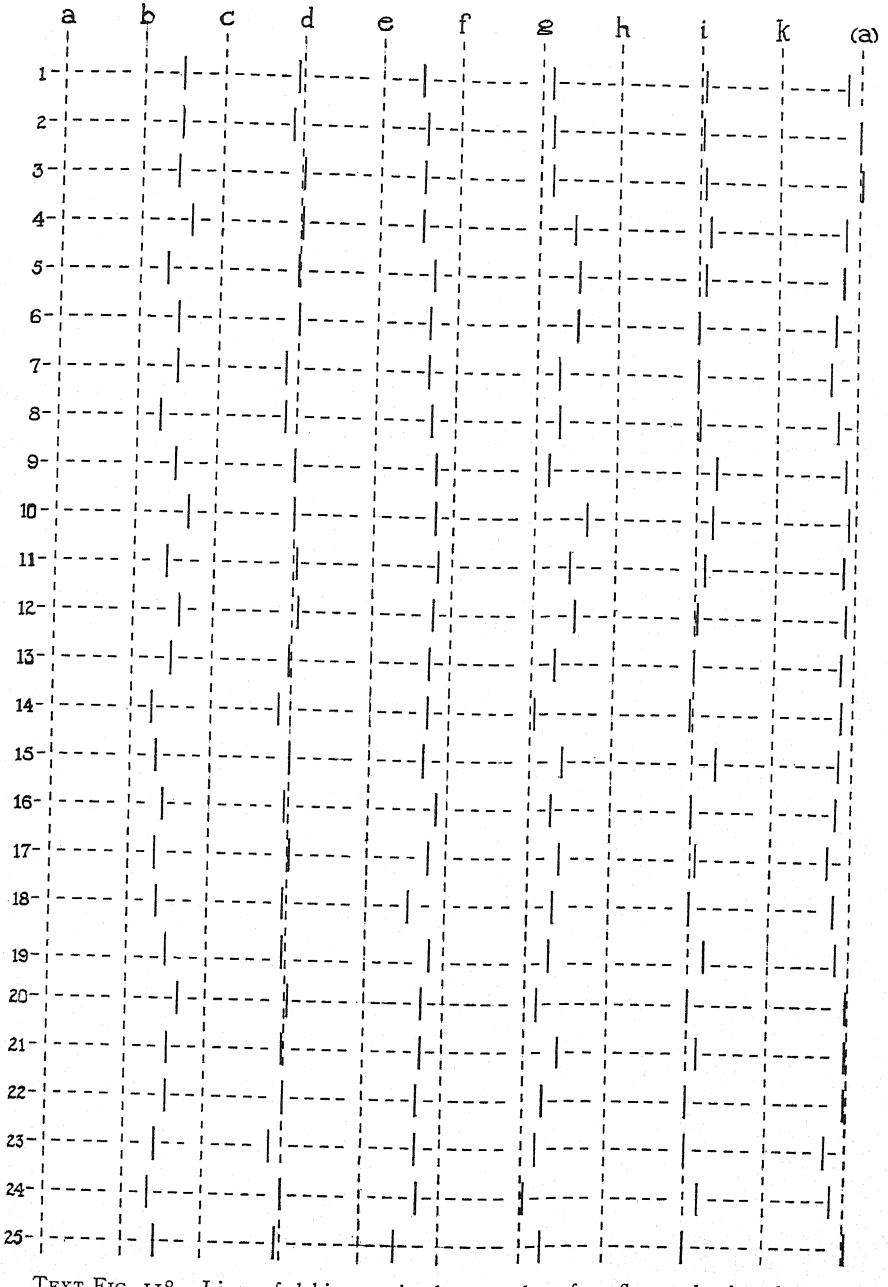
Figures 117 and 118 show the position of the lines of dehiscence of the fruits with reference to the sepals in 50 flowers. In figure 117 the ten positions, *a–k*, of figure 1 are plotted; in figure 118 the ten positions, *a–k*, of figure 2 are similarly plotted. Then, in each of these figures the lines of dehiscence of the capsules of twenty-five flowers are given. It is evident at once that the lines of dehiscence (and presumably the styles) do not occur directly opposite position *g* of these figures; in other words a carpel does not tend to be directly opposite sepal 2 as Wydler, Eichler, etc., have represented it. Instead, this carpel is just a little to one side of position *g*. There is, however, a carpel nearly or directly opposite position *d*, and the other two carpels are symmetrically oriented with reference to this one. The results are practically identical, whether the sepals are arranged as in figure 1 or as in figure 2. The orientation of the carpels is apparently not absolutely fixed, with reference to the sepals, but is fairly constant. The position of the carpels is given in figures 5–109 on the basis of these observations. Several hundred fruits were examined with this in mind—of which figures 117 and 118 show fifty. While this difference in the carpel positions from the previously published figures may seem slight and is not absolute, it is of significance in that the symmetry relations within the flower are dependent, in part at least, on the orientation of the carpels.

That the number of stamens in a flower does not materially affect the position of the carpels has been determined by studying the splitting of the capsules in flowers with various numbers of stamens. Although a flower is occasionally found with the carpels oriented as in Eichler's diagram, this is not the rule—they are usually as in the accompanying diagrams. None of the parts of the flower are fixed in position with absolute rigidity; thus the distance between stamens also varies slightly; sometimes when one stamen is missing the neighboring ones are slightly closer together.

In *S. aquatica* there are typically five carpels arranged in positions *b*, *d*, *f*, *h*, *k*, figure 119,—that is, alternating with the five sepals. It has been

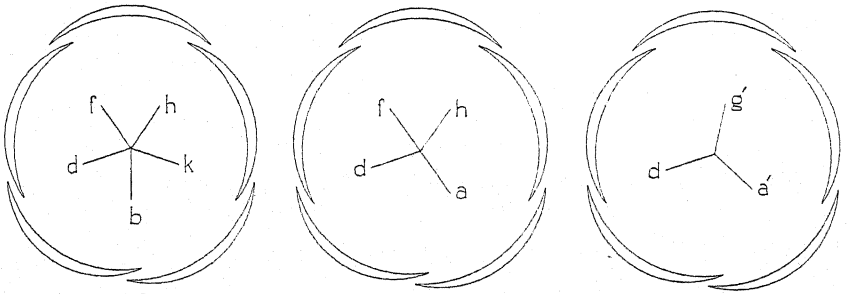


TEXT FIG. 117. Lines of dehiscence in the capsules of 25 flowers having the sepals overlapping as in figure 1; the letters correspond with the positions designated by the letters *a–k* in figure 1.



TEXT FIG. 118. Lines of dehiscence in the capsules of 25 flowers having the sepals overlapping as in figure 2; the letters correspond with the positions designated by the letters *a–k* in figure 2.

shown previously (1929) that when one of the carpels is missing in *S. aquatica*, the four remaining assume the positions indicated in figure 120—that is, the two carpels at *b* and *k* tend to be replaced by one at *a*. In *S. media* there are just three carpels, and apparently the two in positions *f* and *h* are replaced by a single one in position *g'*, that at *a* shifting slightly to position *a'* (fig. 121). This makes a very gradual series—without the



TEXT FIG. 119. Axes *b*, *d*, *f*, *h*, *k*, of the five carpels in a normal flower of *Stellaria aquatica*. TEXT FIG. 120. Axes *d*, *f*, *h*, *a*, of the four carpels of a four-carpeled flower of *Stellaria aquatica*. TEXT FIG. 121. Axes *d*, *g'*, *a'*, of the three carpels in a normal flower of *Stellaria media*.

more violent readjustments necessary to attain the Wydler-Eichler orientation, if, as is generally supposed, the three-carpeled form has been derived from the five-carpeled by reduction. This interpretation seems to fit the facts adequately. Both stamens and carpels, then, show a reduction; stamen reduction is taking place, and carpel reduction has apparently occurred. The three carpels are equidistant from each other. However, in a three-stamened flower, the three stamens are not separated by similar distances. To have the three stamens at equal distances from each other, two would have to be in positions not occupied by stamens in the ten-stamened flower. This apparently does not occur, and in this respect the stamens differ from the carpels. The orientation of stamens and carpels in a three-stamened flower is "economical" considered in the light of the space relationships of the older authors; that this economy may be an expression of minimum surface, and correlated with surface tension phenomena seems possible.

While the usual number of stigmas is three, eighteen of the 4652 flowers examined had four stigmas, and one flower had two. While not enough flowers with four stigmas were found to permit definite conclusions as to the positions of the carpels, they seem to be similar in position to those in similar flowers of *S. aquatica*. In *S. media typica trichocalyx* (1930), in contrast to *S. media neglecta typica*, flowers with two stigmas occurred more frequently than flowers with four. Normal flowers, with three carpels,

have capsules which split into six valves; when there are four stigmas, however, the capsules may open by eight valves, or they may open by only six.

Discussion and Conclusions

The data presented in the preceding pages illustrate the character and range of the numerical and spatial variations in the parts of the flowers of *S. media neglecta typica.* A consideration of these variations, and especially of the position of their occurrence in the flower, brings out immediately the fact that they are not nearly as haphazard as might be supposed. Thus supernumerary petals and supernumerary petaloid stamens occur in the majority of cases in definite positions in the flower; and petaloid stamens or petals apparently in place of stamens are almost restricted to one position. While these irregularities occur, they are rather definite in the place in the flower that they may occupy, and all more or less limited to the one position (*c*). If the "genetic spiral" of the older authors is accepted this is just the place in which such changes would be expected to appear, if a previous spiral arrangement and reduction from more numerous floral parts are postulated.

The stamens show by far the greatest variation both in number and in arrangement. The normal, apparently original, and most frequent number of stamens is ten, arranged in obdiplostemonous fashion opposite the sepals and petals; this is the number most frequently found in the first flowers on a plant. It has been shown by various writers that in *S. media* the variation in the number of stamens is correlated with available food supply and those conditions which affect the metabolism of the plant. Thus variegated plants at first show fewer stamens than vigorous green plants of *S. media typica trichocalyx;* in the same variety it has been shown that removing the flowers after blooming and thus preventing seed-setting, and cutting back plants that have been flowering, also cause an increase in the number of stamens, very ostensibly by decreasing the drain on the food supply which the maturing of seeds entails. In general the larger varieties of *S. media* have a larger number of stamens, and the flowers formed near the beginning (though sometimes not at the very beginning) of the flowering period have more stamens than the later formed flowers. Nourishment, in a broad sense, plays a part in determining the number of stamens.

Perhaps it also plays a part in determining their position; for this, however, there is no evidence. The possibility of correlating the disappearance of the stamens with the theory of reduction from a type with numerous floral parts, spirally arranged, has been taken up; according to the Schimper and Braun spiral, the disappearance, except in the case of the nine-stamened flowers, shows no great regularity; according to the scheme given in figure 4, however, the petaline stamens seem to disappear from the uppermost one downward, the sepaline from the lowest one upward. Too

much emphasis should not be placed on these "spirals" however, since the real evidence for their existence is very limited. If the flowers of this order have come from a primitive type with more numerous stamens and pistils, as is often supposed, it is not known which sporophylls were lost before the present ten-stamened form was evolved.

The ten-stamened flower of *S. aquatica*, with parts in multiples of five, is radially symmetrical. When the stamens and carpels become reduced in *S. media*, the expression of complete symmetry is usually no longer possible, since the stamens are limited in their distribution to the ten positions that they occupied in the ten-stamened type. Even in flowers with fewer than ten stamens, a bilateral symmetry is often achieved as shown in the floral diagrams. The symmetry of these flowers is, in part at least, an expression of surface tension phenomena; these can be realized, however, only within the limits set by inherited tendencies. Reduction in the number of flower parts is very obviously going on.

Examination of figures 5–116 shows that the Hofmeister theory that available space in the flower is the determining factor in orientation of floral parts sometimes does not hold. Thus among others, figures 51, 54, 72, 77, 78, 79, 82, 83, 85, 86, 88, 89, 92, 96, 97, 98, 99, 103, 104, 105, and 107 all have the stamens arranged in a fashion that does not make the most economical use of the available space. Symmetry patterns and economy in the utilization of available space usually go hand in hand. Hofmeister also admitted the rôle that equilibrium in the sense of balance between parts may play.

The evolution of whole lines of development in the plant kingdom is based, to no small extent, on the reduction in the number of stamens and pistils, notably in the order of the Chenopodiales. In the species and variety under consideration a similar reduction, at least in the stamens, takes place not merely in the same species or variety but actually on one plant. That nourishment may have played a part in the evolutionary development in this alliance as it does in the individual plants of *S. media* seems possible.

Highly specialized organs of complex mechanism, that demand perfection in order to be of use to the living individual, cannot, as assumed in the theory of natural selection, be conceived of as passing through a long series of changes, throughout which they would remain useless. In the flowers of *S. media*, the symmetrical three-stamened form is not derived directly from the five- or ten-stamened form through any absolute series of symmetrical types. There are intermediate forms with four, six, seven, etc., stamens that are not symmetrical; however, in spite of this, it has been shown that the four-, six-, and seven-stamened flowers in many cases give an indication in the arrangement of their stamens of the ultimate types that are to be attained; and the intermediate types are frequently not symmetrical nor in any obvious way more effective by this suggestion of the

final type. This is a good example of evolution involving increased delicacy of response to the internal environment. *S. media* gives concrete evidence as to the nature of the laws of variation. Although the range of variations in this species is great, it is limited by such factors as heredity, nutrition and symmetry relationships, and because of this we are able to recognize *Stellaria media*, and the varieties of which this species is constituted.

Summary

In *Stellaria media neglecta typica:*

1. Additional petals and petaloid stamens as well as apparent substitution of petals for stamens, and petaloid for normal stamens, all take place most frequently in one position in the flower (position *c*).

2. Petaline stamens, especially the one in position *f*, are most frequently lost; stamens opposite the two outer sepals are also frequently missing.

3. The ten-stamened flower (figs. 5 and 6) is by far the most abundant of this variety; from this reduction takes place, giving, through various intermediate stages, a five-stamened flower (fig. 87), and a three-stamened type (fig. 108), both of which occur relatively frequently.

4. Flowers with two to ten stamens may all occur on the same plant.

5. The position of the carpels as found in the flowers studied differs slightly from that published in the older works and is apparently correlated with their reduction in number from five to three.

6. The occurrence of supernumerary petals, as well as the disappearance of the stamens, have been considered from the standpoint of the existence of a possible original "spiral" arrangement within the flower; according to one of these "spirals," the microsporophylls disappear from the uppermost one down and from the lowermost one up; those nearer the center of the axis are much less frequently lost.

7. Flowers that approximate bilateral and radial symmetry are much more common than those that are wholly asymmetrical; that surface tension phenomena prevailing during flower development may play some part in determining the distribution of the flower parts seems possible; but that the reduction of floral parts limits the expression of these symmetries seems unquestionable.

I feel indebted to Professors C. C. Curtis and R. A. Harper for their stimulating suggestions and criticisms.

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STOMATAL APPARATUS OF THE CULTIVATED CRANBERRY *VACCINIUM MACROCARPON*

WM. H. SAWYER, JR.

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During studies upon carbohydrate production in leaves of cultivated cranberry varieties, certain previously unrecorded facts relating to the stomata and guard cells were observed; and since it appears that little attention has been paid to these structures in the Ericaceae, particularly in the subfamily Vaccinioideae, it seems worth while to make the following record of the features observed.

NUMBER AND DISTRIBUTION OF THE STOMATA

The thick, narrowly-elliptical leaves of the cranberry plant are very small (2 to 3 mm. broad and 5 to 8 mm. long in the Early Black variety). They occur almost entirely upon the vertical offshoots which arise from the long horizontal runners; it is, of course, the distal portions of these "uprights" which bear the leaves of the current season, while the basal parts are clothed with the preceding season's foliage.

Leaves for stomatal study were selected as follows: from any upright, chosen at random, collections were made from the tip, middle, and base of the current season's growth, and from old leaves of the preceding year. For a given variety, ten leaves were collected from each of the four areas, each leaf from a different upright. The leaves were fixed in absolute alcohol as collected, and two days later were transferred to a mixture of equal parts of 95 percent alcohol and glycerine. To prepare the epidermis for stomatal count, small pieces were shaved off with a safety razor blade, mounted in a mixture of glycerine and alcohol tinted with Brom Phenol Blue, and examined under a four millimeter objective. Accurate counting was facilitated by placing cross hairs in the 10 × ocular to divide the field into quarters, and by using a mechanical tabulator for registering the numbers. The diameter of the microscopic field with tube length at 150 mm. was .382 mm., and its area was .114 sq. mm. As many fields were counted, from different leaves, as seemed necessary to give a true average, from which the total number of stomata per square millimeter of leaf surface was computed.

All stomata are located on the under side of the leaf. The midrib, the petiole, and a narrow zone bounding the periphery of the blade are free from openings, but with these exceptions stomata occur profusely through-

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out the lower epidermis. Their distribution, however, is somewhat uneven. In all leaves stomata are slightly more numerous along the border of the peripheral free area than nearer the midrib. Moreover, in young leaves (leaves of the current season) the stomata are most numerous per unit area at the tips and the younger the leaf, the greater is this number in comparison with other regions. This may be noted by reference to table 1,

TABLE 1. *Average Number of Stomata per Square Millimeter in Cranberry Leaves*

Variety	Tip Leaves			Middle Leaves			Basal Leaves			Old Leaves		
	Tip	Middle	Base	Tip	Middle	Base	Tip	Middle	Base	Tip	Middle	Base
Early Black	871	731	624	763	666	587	754	649	587	570	570	570
Pride	736	745	666	728	675	640	587	578	561	605	570	456
McFarlin	728	684	596	640	640	631	605	570	509	570	491	456
Howes	684	640	535	684	649	631	605	596	631	596	596	596

General Average	Tip Leaves	Middle Leaves	Basal Leaves	Old Leaves
Early Black	742	672	663	570
Pride	716	681	575	577
McFarlin	670	637	561	506
Howes	620	654	610	596

where it will be seen that in the Early Black variety, for example, the topmost leaves, which are the youngest of the foliage, have an average of 871 stomata per square millimeter at the tip as compared with only 624 at the base, while in the oldest leaves of the same variety the number is the same (570) for both tip and base of leaf. The explanation for this difference lies in the fact that as the leaf matures, the tip is the last part of the blade to reach full expansion, and therefore the stomata in this area are not as widely spaced as in the more mature portions of the leaf.

Reference to the table will also show that the average number of stomata per square millimeter for the whole leaf is greatest for the topmost leaves, and in general becomes progressively less in leaves toward the base of the upright. This difference is due to the same condition outlined above, that is, the topmost leaves are the youngest, and because they have not reached full expansion, the stomata are more crowded than in the older, lower leaves. Rea (3) has called attention to a somewhat similar condition in *Campanula*, where the number of stomata per square millimeter on the upper leaf surface was found to increase with higher position on the shoot, and on the under surface with increase of illumination and dryness of habitat. It is difficult to understand how differences in light intensity or other external factors can affect the number of stomata, since they are already formed in the bud and their number for the leaf determined before the blade is exposed to environmental influences. It seems possible, therefore, that in *Campanula* also the number per unit area is determined by the degree of expansion of the leaf blade.

The total number of stomata per square millimeter of leaf surface, in comparison with other plants, is extremely high in cranberry leaves. Weiss (7) counted the stomata in one hundred and sixty seven species of flowering plants (which included only one ericaceous species, a *Rhododendron*) and found that for the under surface of the leaf, the number ranged from 625 in *Olea Europea* to 12 in *Rhipsalis crispata;* and in the upper epidermis from 309 in *Brassica palustris* to 7 in *Tradescantia subospera.* Other plants than the olive which were found to have large numbers of stomata per unit area were *Celtis occidentalis*, 616 per square millimeter, *Fraxinus tamariscifolia*, 600, *Acer platanoides*, 550, and *Morus alba*, 480. Comparison of these plants show that number of stomata is in no way correlated with size or structure of the leaf; certainly the leaves of cranberry, olive, maple, and mulberry have little similarity in size or texture. It should be noted, however, that in all these plants with high stomatal count, the openings are restricted to the epidermis on the under side of the leaf. The average number of stomata in cranberry leaves for the four varieties studied, young and old leaves taken together, is 632 per square millimeter. This exceeds Weiss's figures for the olive, and is the highest count that the writer has found recorded for any plant. In a recent paper on stomata in citrus leaves, Reed and Hirano (4) have found average numbers in the tip, middle, and base of lemon leaves which give a total average for the leaf of 623, very near the average for cranberry leaves. Examination of *Vaccinium oxycoccos*, the wild "moss-cranberry," indicates that this species has 520 stomata per square millimeter on the old leaves, a lower count than for the varieties of *V. macrocarpon* studied with the exception of the McFarlin variety. Gleisberg (1) states that in Europe there are 6 to 10 stomata per linear 100 μ in *V. oxycoccos*, which would make the number per square millimeter considerably larger than found by the writer.

When comparison was made of the stomata and guard cells of different varieties, it was found that these structures in the Early Black, Howes, and McFarlin varieties are similar in size, with guard cells that average 21.75 μ in length with an average breadth of 18.0 μ across the two cells. Guard cells of the Pride variety are slightly larger, 22.35 μ by 19.35 μ; while in *V. oxycoccus* the measurements 21.0 μ by 18.75 μ, indicate that the pair of guard cells forms a slightly more rounded unit than in the other types. In the varieties studied there is no measurable difference in the stomatal slit, which averages from 5 μ to 7 μ in length and, when fully opened, 2 μ in breadth.

Examination of leaves of the high bush blueberry, *V. corymbosum*, disclosed that here also the stomata and guard cells are restricted to the under surface and that in structure, size, and number they closely simulate comparable structures in the cranberry leaf; indeed, the stomatal apparatus in the two plants is so similar as to be indistinguishable, although the other leaf structures have well-defined differences.

Structure of the Stomatal Apparatus

The general orientation of the guard cells is lengthwise of the leaf; many, however, are placed more or less obliquely, and occasionally a pair will be found with the long axis of the cells crosswise of the blade. The stomata never open wide, and as a result opposed faces of the guard cells form nearly a straight line. The outer surfaces are typically crescentic, however, and the two cells form an elliptic-oval shaped unit. The walls are convex and much thinner than in the adjoining epidermal cells. The nuclei average about 6 μ in diameter, which is about two-thirds the diameter of the whole cell, and they are spherical or oval in shape (Pl. XLI, fig. 1).

The ordinary structure cells of the epidermis should receive brief mention at this point. They form a distinct pattern with the guard cell units, in which the latter are bounded on either side by a narrow epidermal cell, concaved to fit around the guard cell, and the transverse row thus formed alternates with a row of larger, elongate epidermal cells, which have their long axes crosswise of the leaf; this row contains no guard cells. All these structure cells of the epidermis have thick walls with prominent middle lamellae and simple pits which are most numerous in the walls parallel to the long axis of the guard cells. The nuclei are slightly smaller than those of the guard cells. These details may best be seen in figure 2.

The wall of the guard cell, as seen from the external surface, is uniform in its appearance (fig. 3). When viewed from the side adjacent to the chlorenchyma, however, the ends of the guard cells at a certain depth of focus appear as though distended into pouches (fig. 4); this appearance is misleading and is due, not to a distension of the guard cell ends, but to a thickening of the cell wall. It is interesting that this pouch-like appearance closely simulates the "polar vesicles" described for *Casuarina* by Rehfous (5), and the appearance of the guard cells of *Psilotum* described by Zimmermann (8), but these investigators found an actual distension of the ends of the guard cells, connected by a narrow canal, whereas in the guard cells of *Vaccinium* the lumen is uniform in diameter throughout its length.

The stomatal opening is best studied in median cross sections through the stomatal apparatus. In such sections the guard cells are asymmetrically pear-shaped with relatively thin cellulose walls and homogeneous, finely granular protoplasmic content (figs. 5, 6, 7, and 8). Where each guard cell joins the inner wall of the adjacent structure cell, there is a thickening of the cellulose, which appears in transverse section as a slightly overhanging projection; this pair of thickened areas, one on either side, thus forms a gateway between the stoma and the adjacent intercellular air chamber. This "gate," it should be noted, is always open, as is the slit-like opening in the cuticle on the outer surface of the epidermis, and the opening or closing of the stoma is entirely determined by change in shape of the guard cells, which, when rounded out by increased turgor, press firmly together and close the stoma.

The intercellular air chambers are comparatively small in cranberry leaves and may be restricted to a narrow space above the stoma between the inner walls of the epidermal cells (fig. 8).

Microchemical tests of thin, freshly-cut sections with chlor-iodid of zinc gave a deep yellow color in the thick outer walls of the structural cells of the epidermis, indicating heavy cutinization, while the inner and cross walls of these cells, and the walls of the guard cells, gave the blue reaction for cellulose.

The distribution of chloroplasts in the epidermis is noteworthy. Guard cells, the usual seat of plastids, never contain chloroplasts in the cranberry leaf. The other cells in the lower epidermis do, however, contain protoplasmic bodies which in size and appearance are identical with the chlorenchyma plastids (figs. 5, 6, and 8). Iodine solution did not give the blue reaction for starch in these structures, but their similarity to the chlorenchyma plastids, and the fact that like bodies are found in the upper epidermal cells that do give a starch reaction with iodine, seems good evidence that they are true chloroplasts.

Behavior of the Stomatal Apparatus

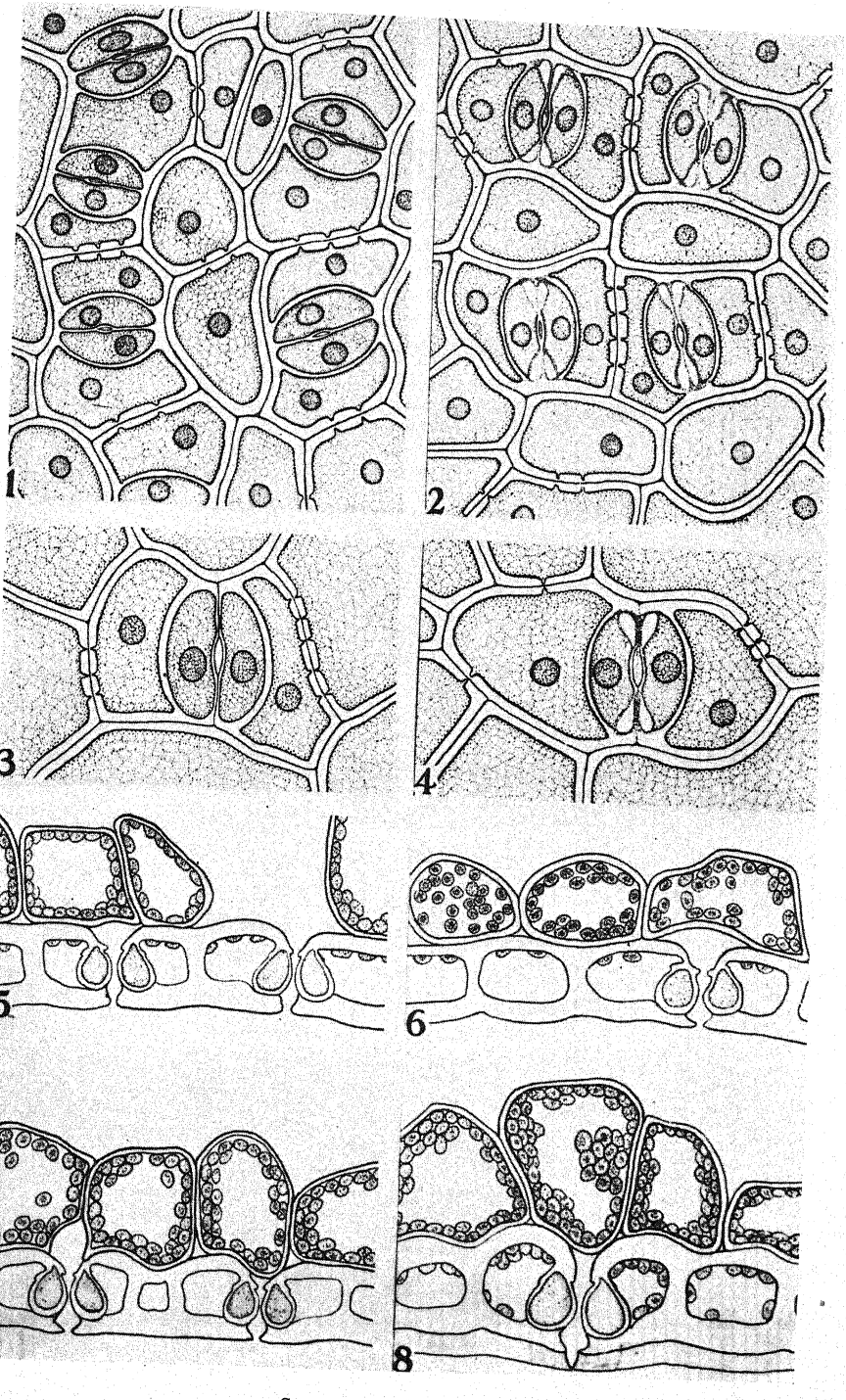
Stomata in cranberry leaves never open widely, as they do in the potato and other plants studied. The behavior of the guard cells in regulating the stomatal openings is very erratic; under identical conditions the guard cells not only in different varieties, but even in the same leaf, may act very differently, some of the stomata being open while others are tightly closed. Sayre (6), Loftfield (2) and others have found in several plants that stomata tend to close at midday, and in a general way the guard cells in cranberry leaves function similarly. This behavior, it may be noted, is correlated with photosynthetic activity, which ordinarily reaches its peak in the late forenoon. In general it would appear that the stomatal apparatus in cranberry leaves is poorly adjusted to changing conditions of light, temperature, and moisture, and responds slowly or sometimes not at all to these environmental factors.

About seventy-five varieties of *Vaccinium macrocarpon* Ait. are in cultivation in the different commercial cranberry areas in this country at the present time. Of these many varieties, the four chosen for study represent quite different types, and it was hoped that differences might be found in the stomatal apparatus which would further emphasize their divergence. It does not seem, however, that the variations in number, structure, or behavior of the stomata and guard cells are great enough to have any taxonomic or physiologic significance.

Summary

1. The stomatal apparatus was studied in four cultivated varieties of the cranberry, *Vaccinium macrocarpon.*

2. No differences of taxonomic or functional significance occur among the four varieties.



SAWYER: CRANBERRY

3. The average number of stomata per square millimeter is 632, which seems to be the highest number ever recorded for any plant. Stomata occur only on the under side of the leaf.

4. No chloroplasts are found within the guard cells, but chloroplasts do occur in the other epidermal cells on both sides of the leaf.

5. The stomatal apparatus is poorly adjusted in function to changing environmental factors of light, temperature, and moisture.

6. The stomatal apparatus in *V. macrocarpon* and in the high-bush blueberry, *V. corymbosum*, is identical in every respect.

This work was done in the course of investigations on cranberry problems for the Massachusetts Agricultural Experiment Station, at the cranberry station at East Wareham, Massachusetts. To Dr. H. J. Franklin, in charge, the writer expresses appreciation for his guidance and unfailing interest.

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DESCRIPTION OF PLATE XLI

FIG. 1. External surface of lower epidermis of cranberry leaf. The shape, comparative size, and structure of the guard cells and structure cells may be noted, also comparative thickness and pitted character of the walls. × 650.

FIG. 2. Lower epidermis, as viewed from inside. Note particularly the thickened areas at the ends of the guard cells. × 650.

FIG. 3. Guard cell unit and adjacent cells, showing features of figure 1 in greater detail. × 850.

FIG. 4. Detail of figure 2. × 850.

FIG. 5. Cross-section through lower epidermis and adjacent chlorenchyma cells of young leaf of Early Black variety, showing two guard cell units and their stomata. Note the chloroplasts in the spongy parenchyma cells, and similar bodies in the epidermal cells; also absence of these structures in the guard cells. × 650.

FIG. 6. Cross-section through young leaf of Howe variety; structural features as above. × 650.

FIG. 7. Cross-section through old leaf of Early Black variety. × 650.

FIG. 8. Cross-section through old leaf of *Vaccinium oxycoccus*. × 650.

ON THE NUTRITION OF THE CULTIVATED MUSHROOM, *AGARICUS CAMPESTRIS*, AND THE CHEMICAL CHANGES BROUGHT ABOUT BY THIS ORGANISM IN THE MANURE COMPOST [1]

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In the study of the nutrition of the cultivated mushroom, *Agaricus (Psalliota) campestris*, and the chemical changes that are brought about when this organism is cultivated upon composted horse manure, two definite processes must be recognized: 1, the transformations of the various organic and inorganic constituents of the manure by the numerous micro-organisms during the process of composting; 2, the transformations of the organic and inorganic constituents of the compost when it is placed in the mushroom house and inoculated with the mycelium ("spawn") of the mushroom, from the time of "spawning" until the residual manure is finally discarded as "spent manure"; these changes are due primarily to the nutrition of the mushroom fungus itself. It is essential to obtain this information before an attempt can be made to develop an artificial manure, or a compost prepared entirely from plant residues and inorganic fertilizers, without depending on the excreta of the horse or other animals.

A detailed review of the earlier literature on the growth and nutrition of the mushroom is found in the work of Hoffmann (6, 7), Ferguson (1*a*), Repin (9) and Hein (3). Styer (10, 11) has shown that the mycelium of the mushroom will grow on a variety of substances, including sugars, hemicelluloses, cellulose, lignin and proteins. Hein (4, 5) and Lambert (8) found that artificial composts can be readily prepared, which will allow the mushroom to grow and reach a marketable size. Falck (1) prepared a manure compost by the "hot fermentation" process, which was found to lead to a destruction of the cellulose and to an enrichment in the lignin content. When the mushroom fungus is inoculated into this compost, it was found to bring about a process of corrosion, the latter term being used to designate the decomposition by the organism of both the cellulose and the lignin. Falck suggested that the process of preliminary composting of the manure must be interrupted in time so as to leave a certain amount of cellulose for the needs of the corrosive fungus. Waksman and McGrath (13) traced the chemical changes that take place in the composting of

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manure and during the growth of the mushroom, and found marked differences in the chemical changes brought about by the microörganisms during the first and second stages of the decomposition of the manure and the manure compost. Significant losses of cellulose and lignin were observed as a result of the growth of the mushroom, accompanied by marked increases in the water-soluble substances. Recently Waksman and Nissen (14) demonstrated that the lignin and the protein of the compost form the most essential nutrients in the growth of the cultivated mushroom.

The following studies were outlined for the purpose of establishing: 1, the changes brought about in the chemical composition of manure composts at different stages of the development of the compost, and especially as a result of the growth of the *Agaricus campestris;* 2, the chemical changes taking place in specially prepared composts, which have been kept under constant conditions with careful records regarding the losses in weight and the temperature changes, both during the process of preliminary composting and during the growth of *Agaricus campestris* in the mushroom house; 3, the chemical changes produced in the organic constituents by *Agaricus campestris* grown in pure culture, in bottles, on sterilized fresh and composted manure. The methods of analysis used in these studies were those described by Waksman and Stevens (15).

Chemical Changes Produced in Composts of Horse Manure by the Growth of Agaricus campestris

For this study a series of five samples were obtained from manure composts, at different stages of decomposition, and from composts in which the mushroom was growing, from each of three mushroom growers. It is assumed that the manure used by each grower was the same in the case of all the composts, from which the five different samples were obtained. However, an appreciation of the variability of the manure obtained through an agent from city stables leaves much to be expected in this connection; further, the fact that in some cases the fresh manure was used immediately when brought from the stable for the preparation of the compost, and at other times the manure may have been kept in large heaps for many months, will serve further to increase the variability of the material used. With all these limitations in mind, the changes observed in the chemical composition of the manure, as a result of composting and as a result of the growth of the mushroom in the compost, will be found to be quite significant. The sources and nature of the fifteen samples of manure used in this experiment are briefly summarized in table 1.

The results of analysis of the proximate chemical composition of the various samples are given in tables 2–4. These results show that the chemical composition of the manure varied in the three houses; the manure used in series *C* had a considerably higher ash content and a much lower nitrogen content than the other manures. Where the nitrogen content was

TABLE 1. *Description of Composts from Which Samples were Obtained*

Source of Manure	No. of Sample		Moisture Content of Fresh Material, Percent
		Series A	
Willits & Son*	1	Manure pile, 3 weeks after third turning, about to go into the mushroom house	59.7
" "	2	Bed spawned 2 weeks, no spawn included in sample	54.6
" "	3	Bed spawned 4 weeks, with spawn included	40.8
" "	4	Bed at second break of mushrooms	44.1
" "	5	Bed at third break, house continued to yield a crop 2–3 months longer	37.6
		Series B	
Chester Co. Laboratories*	6	Bed spawned 2 weeks, no spawn in sample	47.8
" " "	7	Bed spawned 2 weeks, with spawn in sample (same bed as sample 6)	42.1
" " "	8	Bed after 5 weeks of mycelium development	41.3
" " "	9	Bed with 8 weeks of mycelium development, just beginning to bear mushrooms	43.3
" " "	10	Bed bearing 2 months, continued to bear 3 months longer	37.2
		Series C	
E. J. Jacob, Inc.*	11	Manure pile, at time of filling beds	55.3
" " " "	12	Bed 2 weeks after spawning, no spawn in sample	44.7
" " " "	13	Bed spawned 5 weeks, with mycelium	39.8
" " " "	14	Bed at 2nd break of mushrooms	49.9
" " " "	15	House nearly finished	41.0

* Manure used in all the five samples is from the same source, and presumably the same.

TABLE 2. *Chemical Composition of Samples in Series A. Percent Basis of Dry Material*

No. of Sample	Water-Soluble Nitrogen		Total Nitrogen	Hemicelluloses	Cellulose	Lignin	Ash
	Total N	NH_3-N					
1	0.254	0.009	2.757	7.93	12.94	29.99	27.13
2	0.204	0.037	2.606	5.57	7.18	32.05	44.32
3	0.328	0.088	2.206	6.57	8.69	21.80	42.38
4	0.346	0.076	1.729	5.10	4.49	22.59	48.96
5	0.368	0.077	2.205	4.58	3.89	24.90	49.89

TABLE 3. *Chemical Composition of Samples in Series B*

No. of Sample	Water-Soluble Nitrogen		Total Nitrogen	Hemicelluloses	Cellulose	Lignin	Ash
	Total N	NH_3-N					
6	0.185	0.003	2.497	7.35	13.15	31.89	26.19
7	0.304	0.021	2.316	6.27	11.96	27.39	33.13
8	0.396	0.085	2.594	7.27	9.08	28.35	32.98
9	0.517	0.121	2.037	6.08	6.62	23.96	49.59
10	—	—	1.444	3.87	4.74	21.91	62.74

TABLE 4. *Chemical Composition of Samples in Series C*

No. of Sample	Water-Soluble Nitrogen		Total Nitrogen	Hemicelluloses	Cellulose	Lignin	Ash
	Total N	NH_3-N					
11	0.187	0.002	1.474	6.01	13.82	23.95	44.79
12	0.159	0.009	1.745	5.10	7.68	24.47	51.15
13	0.174	0.054	1.635	3.79	6.17	21.60	58.58
14	0.379	0.052	1.757	3.80	6.06	21.85	53.90
15	0.279	0.023	1.717	2.87	5.06	16.57	59.05

low at the beginning, as in series *C*, there was a gradual increase in total nitrogen with the growth of the mushroom. Where the nitrogen was high at the beginning of composting, the relative nitrogen content gradually diminished. It is important to note the rapid increase in the total water-soluble nitrogen and especially of the ammonia with the advance of the growth of the mushroom. During the most active stage of mycelium development, when the mushrooms were formed, as much as 20 to 25 percent of the total nitrogen in the bed was in a water-soluble form. The ammonia made up an important part of this water-soluble nitrogen at this stage of mushroom growth.

As to the organic constituents of the compost, the following changes are noted: during the first two stages, when the mycelium of the mushroom has not developed as yet or only to an insufficient extent, there is a decrease in the cellulose and hemicellulose content and an increase in lignin content. The dry weight of the manure was thereby gradually reduced, as shown by the increase in the relative ash content. However, when the mushroom development became extensive, as during the third and fourth stages, there was a gradual reduction in the lignin content. Since the ash content continued to increase, one may conclude that although the organic matter of the bed was decreasing in total amount, the relative lignin content was decreasing even relatively, *i.e.* that as a result of the extensive development of the mushroom, the most notable change recorded from these results is a reduction in relative as well as absolute content of the lignin-like materials in the composts. These results are in agreement with the results reported by Hébert (2) as well as those reported previously (13).

However, without being able to record quantitatively the changes in concentration of the individual chemical complexes, it is impossible to conclude from these experiments the nature of the feeding power of the mushroom, and the substances which it attacks primarily. Of course, one could calculate from the relative increase in the ash content the actual reduction of the various organic chemical complexes, but due to the uncertainty of the composition of the original manures, such calculations would not be fully justified.

Preparation of Manure and Straw Composts under Controlled Conditions

In order to follow the changes in the chemical composition of the manure during the process of composting and during the growth of the mushroom fungus, it was essential to prepare composts under controlled conditions and to follow the relative and absolute changes taking place in the different chemical constituents, as well as to study the development of various microörganisms responsible for these changes. Four composts were prepared, weighing about 1000 lbs. each, and placed outside under cover, with the southern exposure open, so as to permit exposure to the sun. These composts were started too late in the season, namely on October 30, when the outside temperature was rather low and when there was already constant danger from frost. The composts were placed close to one another, so as to prevent too much dissipation of heat; when the temperature reached 65–70°, the composts were turned and well mixed. The four composts carried to completion in this experiment can be briefly described as follows:

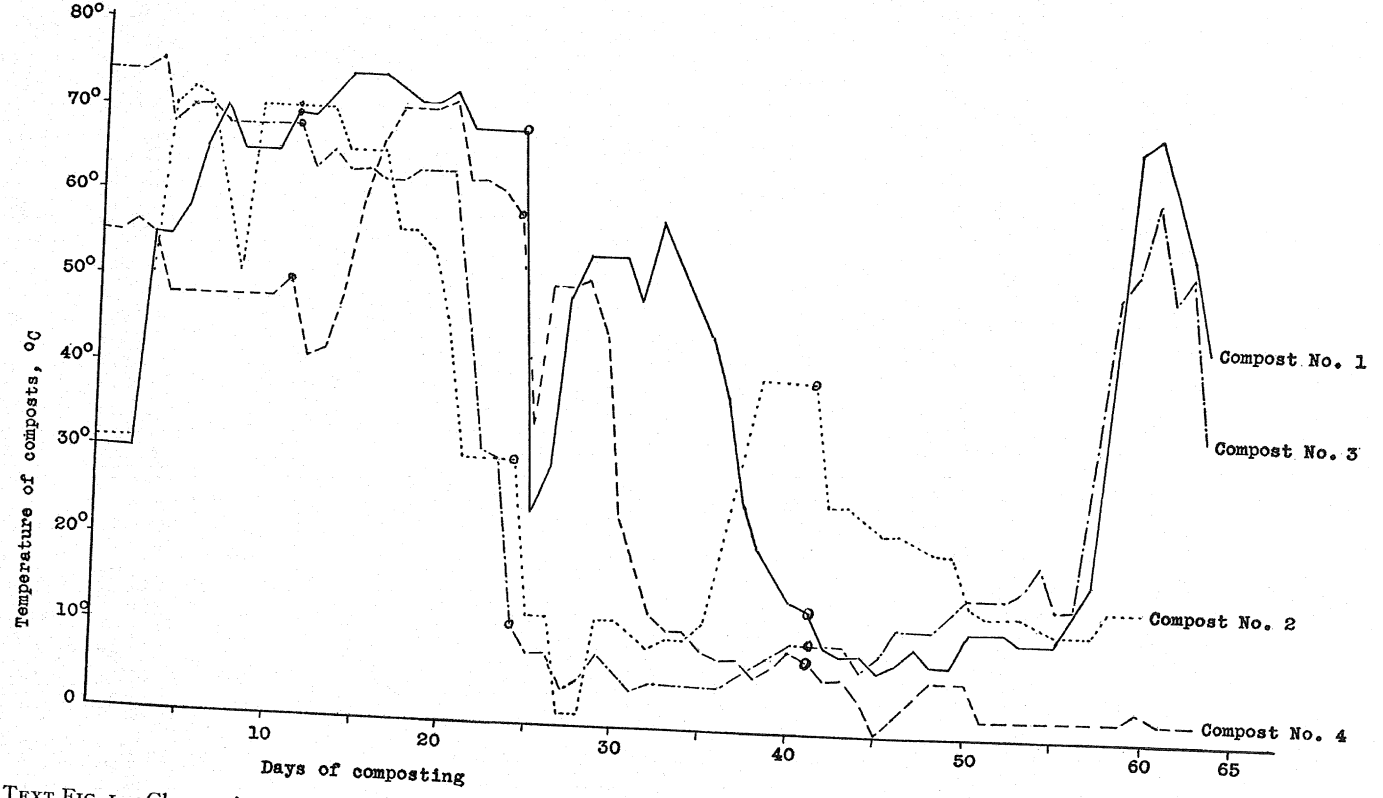
Compost No. 1. Horse manure from New York stables, containing both droppings and bedding, with a total moisture content of about 70 percent. The dry matter content of this compost was, therefore, about 300 lbs. The compost was started immediately after the manure arrived.

Compost No. 2. For the preparation of this compost, 750 lbs. of New York manure and 85 lbs. of wheat straw (25 percent of total dry weight of compost) were used. This compost received 1,462 gm. ammonium phosphate, 1,125 gm. calcium carbonate, and 225 gm. kainit. The salts were mixed with the straw and the latter mixed with the manure. Sufficient water was later added to bring the moisture of the compost to 70 percent. This compost as well contained about 300 lbs. of dry material. Due to the fact that the bedding in this manure consisted of long straw, which could not possibly be mixed uniformly with the droppings, until the first turning, the analyses of the first set of samples from these two composts were rather variable.

Compost No. 3. For this compost, daily collected fresh manure and bedding from the horse stable at the College Farm in New Brunswick was used, so that the manure should be not more than a few days old. Due to the higher moisture content of this manure, the total dry matter was less than in the first two composts.

Compost No. 4. The same source of manure was used as in the case of compost No. 3, except that the bedding was discarded, and only the droppings were collected.

The first mixing of the composts took place after 13 days, the second mixing after 27 days. The composts were carefully watered after each mixing. In view of the fact that a frost had set in by this time, the temperature rose slowly, reaching only 50° C.; the third mixing took place 16 days after the second. Compost No. 4 usually heated up much quicker than



TEXT FIG. 1. Changes in temperature during the composting of the different manure preparations. ————, compost No. 1; ----------, compost No. 2; —.—.—, compost No. 3; – – – –, compost No. 4. O indicates the date of mixing the manure.

TABLE 5. *Changes in Total Weight (in pounds) of the Differently Treated Composts During Decomposition*

No. of Compost	Nature of Compost	Period of Composting									
		At Start		13 Days		27 Days		43 Days		63 Days	
		Moist Weight	Dry Weight	Moist Weight	Dry Weight	Moist Weight	Dry Weight	Moist Weight	Dry Weight	Moist Weight	Dry Weight
1.	Horse manure from New York stables	1000	298	771	301	534	224(?)	527	227	440	224
2.	Three parts of New York horse manure and one part wheat straw	835	296	853	307	574	264	515	247	478	249
3.	New Brunswick horse manure (including bedding)	1000	265	722	217(?)	495	208	510	209	472	208
4.	New Brunswick manure, droppings only	1000	284	902	280	730	234	637	210	580	209

the others. After 63 days of composting the four composts were well mixed and part of each compost placed in mushroom beds (42 × 32 inches in size) using two beds for each compost. The temperature changes in the four composts are given in text figure 1.

The total moist and dry weights of the composts at the beginning, at the various periods when the composts were turned, and at the end when the composts were placed into the beds, are given in table 5. After the third mixing (43 days), there was practically no further decomposition, as shown by the fact that the total dry weight remained more or less constant. Composts No. 1 and No. 3 reached maximum decomposition after 27 days of composting, while compost No. 2, due to the added straw, and compost No. 4, due to its compactness, decomposed more slowly.

TABLE 6. *Chemical Changes Produced in the Decomposition of Compost No. 1 (New York Horse Manure)*

Chemical Constituents	Time of Analysis						
	At Start		27 Days		63 Days		108 Days
	Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration
	%	lbs.	%	lbs.	%	lbs.	%
Ether-soluble fraction	2.87	8.6	1.36	3.1	1.10	2.5	1.05
Cold water soluble organic matter	3.64	10.8	4.72	10.6	3.31	7.4	4.24
Hot water soluble organic matter	1.48	4.4	5.00	11.2	3.89	8.7	2.49
Hemicelluloses	17.78	53.0	15.40	34.5	13.02	29.2	13.59
Cellulose	28.35	84.5	26.32	59.0	15.59	35.0	14.30
Lignin	15.64	46.6	20.44	45.8	27.02	60.6(?)	25.70
Water-insoluble protein	7.81	23.3	11.56	25.9	13.13	29.5	13.63
Total nitrogen	1.44	4.3	1.92	4.3	2.35	5.3(?)	2.38
Ash content	13.48	40.2	14.5	32.5	15.49	34.8	17.20

The changes in the concentration of the various chemical constituents in the composts, both relative and total, are reported in tables 6–9. The total amounts of the various chemical constituents, at the different periods of composting, were calculated by multiplying the total dry weight of the compost at that particular period by the percentage composition of the various constituents. The proximate chemical analyses of the composts were made at the beginning of composting, during the second turning (27 days), when the composts were removed to the mushroom beds, and after the composts had been in the beds for 45 days. In view of the fact that in the last analysis the weight of the compost could not be determined, only the relative concentration is reported in this instance; the spawn was also included in the last sampling.

In making the analyses, samples were collected from several parts of each compost and well mixed. However, due to a proper lack of uniformity of the material, it was impossible to obtain very uniform samples. This

TABLE 7. *Chemical Changes Produced in the Decomposition of Compost No. 2 (New York Horse Manure with 25 Percent Added Wheat Straw)*

Chemical Constituents	Time of Analysis									
	At Start					27 Days		63 Days		108 Days
	Relative Concentration		Total Weight			Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration
	Straw %	Manure %	Straw lbs.	Manure lbs.	Total lbs.	%	lbs.	%	lbs.	%
Ether-soluble fraction	2.55	2.87	1.9	6.4	8.3	1.28	3.4	1.30	3.2	1.34
Cold water soluble organic matter	3.97	3.64	3.1	8.2	11.3	5.45	14.4	3.79	9.4	4.30
Hot water soluble organic matter	1.38	1.48	1.1	3.3	4.4	5.45	14.4	4.02	10.0	2.69
Hemicelluloses	26.76	17.78	20.8	39.8	60.6	17.31	45.7	16.98	42.2	17.13
Cellulose	36.88	28.35	28.7	63.5	92.2	27.19	71.8	19.74	49.1	18.20
Lignin	14.19	15.64	11.1	35.0	46.1	20.00	52.8(?)	25.31	62.9(?)	23.10
Water-insoluble protein	1.06	7.81	0.8	17.4	18.2	11.38	30.0	10.42	25.9	10.66
Total nitrogen	0.31	1.44	0.2	3.2	3.4	1.92	5.1(?)	1.91	4.8(?)	1.83
Ash content	3.29	13.48	2.6	30.2	32.8	12.20	32.2	11.79	29.3	13.70

accounts for some of the discrepancies of the results. A question mark has been placed in all those cases where the accuracy of the determination, due possibly to variation of sample, is questioned. It may also indicate that the accuracy of the particular determination in the previous analysis is open to question. It is sufficient to cite the following illustration. The

TABLE 8. *Chemical Changes Produced in the Decomposition of Compost No. 3 (New Brunswick Horse Manure)*

Chemical Constituents	Time of Analysis						
	At Start		27 Days		63 Days		108 Days
	Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration
	%	lbs.	%	lbs.	%	lbs.	%
Ether-soluble fraction	1.85	4.9	1.28	2.7	1.02	2.1	0.70
Cold water soluble organic matter	4.45	11.8	6.64	13.8	4.49	9.3	5.11
Hot water soluble organic matter	2.95	7.8	7.43	15.4	4.90	10.2	3.85
Hemicelluloses	18.84	49.9	15.26	31.7	12.52	25.9	11.74
Cellulose	27.41	72.6	20.41	42.4	14.08	29.3	12.00
Lignin	17.69	46.9	19.98	41.5	26.69	55.4(?)	22.3
Water-insoluble protein	8.25	21.9	11.50	23.9	13.44	27.9	13.56
Total nitrogen	1.58	4.2	2.05	4.3	2.48	5.2(?)	2.62
Ash content	12.51	33.2	17.8	37.0(?)	22.87	44.4(?)	25.5

TABLE 9. *Chemical Changes Produced in the Decomposition of Compost No. 4 (New Brunswick Horse Manure, Droppings Only)*

Chemical Constituents	Time of Analysis						
	At Start		27 Days		63 Days		108 Days
	Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration	Total Weight	Relative Concentration
	%	lbs.	%	lbs.	%	lbs.	%
Ether-soluble fraction	1.73	4.9	0.97	2.3	0.65	1.4	0.53
Cold water soluble organic matter	3.71	10.5	3.57	8.3	3.23	6.7	5.50
Hot water soluble organic matter	4.21	12.0	5.36	12.5	3.46	7.2	3.66
Hemicelluloses	18.69	53.1	12.50	29.2	11.89	24.8	11.33
Cellulose	22.95	65.2	14.46	33.8	10.45	21.8	10.20
Lignin	20.81	59.1	20.17	47.1	26.10	75.4(?)	22.30
Water-insoluble protein	9.81	27.9	14.19	33.1	13.25	27.7	12.38
Total nitrogen	1.81	5.1	2.30	5.4	2.32	4.9	2.4
Ash content	20.00	56.8	30.3	70.8(?)	32.91	68.7(?)	34.4

total nitrogen increased, in table 6, from 4.3 lbs. at the beginning of the period of composting to 5.3 lbs. at the end of composting; one could hardly feel justified in ascribing this increase of the total nitrogen to nitrogen-fixation, but one may reasonably conclude that it is due to the non-uniformity of the material. The same is true of the total increase in the lignin content, reported in the same table. The absolute increases in the

lignin content may of course be due to the synthesis of lignin-like complexes by the microörganisms active in the decomposition processes. With all these limitations in mind, however, the results will still prove to be very illuminating.

In the process of composting of manure, there is a gradual decrease of the ether-soluble substances, a slow decrease of the cold-water soluble material, a rapid decrease of the hemicelluloses and cellulose and an accumulation of the lignin, water-insoluble protein, and ash. In other words, in the process of composting of manure, changes occur similar to those which were found to take place in the composting of plant residues, such as straw, leaves and corn stalks in the laboratory, in the presence of additional available inorganic nitrogen. The differences in the rapidity of decomposition of the various organic complexes in the several composts is quantitative rather than qualitative. On comparing composts No. 1 and No. 2, it is found that the second compost, which received additional straw, contained more cellulose, more hemicellulose, less total nitrogen, and less water-insoluble protein, as was to be expected. On comparing composts No. 3 and No. 4, the last one being free from bedding, it is found that the latter was richer in total nitrogen and in ash and poorer in hemicellulose and cellulose, as was also to be expected.

Ordinarily, when composts are placed in the beds in the mushroom house, they are allowed to heat up; if the temperature does not rise as a result of the decomposition of the compost by microörganisms, the house is steamed for 24 hours, so as to destroy the injurious insects. In this experiment, however, no steam was available, while the temperature of the composts did not increase through the agencies of decomposition. The beds were spawned 10 or 11 days after the compost was introduced into the bed. The temperature of the beds was then about 20° C. After 35 days, the beds were covered with one inch of soil, the reaction of which was adjusted to pH 6.8–7.0 with $CaCO_3$, and the beds were watered. Thirty days after the beds were covered with soil, the first fruiting bodies of the *Agaricus* appeared. The temperature of the beds was about 18° C.

A careful record was kept of the yields of mushrooms from the beds containing the differently treated composts, allowing about 18 square feet of bed space for each compost. These yields were as follows:

Compost No. 1	10,150 gm.
Compost No. 2	11,547 gm.
Compost No. 3	10,470 gm.
Compost No. 4	13,823 gm.

In view of the fact that the composts were started late in the season and that the cellar used for the growth of the mushrooms was far from ideal for this purpose, the yields are highly satisfactory. The total yield was 101 pounds for about 50 square feet of space, or about 2 pounds per square foot, which is above the common average obtained in commercial

TABLE 10. *Proximate Composition of Spent Mushroom Manure, in Percent of Dry Material*

Compost No.	Ether-soluble Fraction	Cold Water Soluble			Hot Water Soluble Organic Matter	Hemicelluloses	Cellulose	Lignin	Water-insoluble Protein	Ash	Total Nitrogen
		Organic Matter	Total Nitrogen	NH_3-N							
1	0.852	13.25	0.634	0.146	9.28	7.07	11.92	17.12	11.42	23.2	2.47
2	0.367	11.19	0.540	0.110	8.63	8.64	17.62	16.84	7.99	24.8	1.79
3	0.539	13.87	0.864	0.222	8.91	3.88	9.61	12.95	12.54	36.0	2.72
4	0.457	9.68	0.812	0.282	7.53	4.77	7.32	18.09	10.66	50.3	2.28

TABLE 11. *Decomposition of Various Composts by Pure Cultures of Agaricus campestris*

Compost No.	Period of Incubation	Total Dry Weight*	Ether-soluble Portion	Cold Water Soluble Organic Matter	Hot Water Soluble Organic Matter	Hemicelluloses	Cellulose	Lignin	Water-insoluble Protein	Total Nitrogen	Water-soluble Nitrogen		Ash
											Total	NH_3-N	
	days	gm.	%	%	%	%	%	%	%	%	%	%	%
1	Control	100	1.48	4.19	3.53	13.34	20.05	22.08	13.06	2.29	0.330	0.097	16.3
1	104	89.4	1.07	7.21	8.68	10.59	—	19.17	12.75	2.71	0.431	0.080	20.5
1	256	87.1	—	10.10	6.97	10.03	21.93	16.97	12.01	2.43	0.506	0.144	18.5
2	Control	100	1.43	4.64	3.88	15.75	19.38	23.69	11.63	2.12	0.360	0.076	15.1
2	104	85.8	0.86	12.41	7.33	12.36	16.72	13.17	9.44	2.56	0.517	0.306	15.5
2	256	64.3	—	25.78	5.04	5.17	18.84	8.29	20.03	3.41	1.191	0.486	22.7
3	Control	100	1.02	5.93	5.37	12.40	20.64	23.24	12.63	2.31	0.580	0.218	24.3
3	104	96.1	0.68	7.82	7.67	10.58	13.60	15.26	11.50	2.86	0.966	0.466	25.1
3	256	67.9	—	24.46	6.36	2.70	9.61	8.07	20.01	3.40	1.884	0.700	40.8
4	Control	100	0.98	3.72	3.05	12.31	13.72	21.08	11.19	2.03	0.441	0.183	32.9
4	104	90.5	0.49	3.90	2.89	11.63	10.16	15.02	9.38	2.05	0.482	0.278	34.4
4	256	77.8	—	22.34	4.07	2.38	12.63	6.10	17.18	2.94	1.392	0.562	45.4

* Results calculated on 100 gm. basis of control bottles.

houses (1½ pounds per square foot). The largest yield was obtained in Compost No. 4, which was prepared from the pure droppings, free from bedding, but the mushrooms were very small. Compost No. 2, to which 25 percent of straw was added, gave a high yield, and the mushrooms were uniform, all edible, and could readily be classified as marketable.

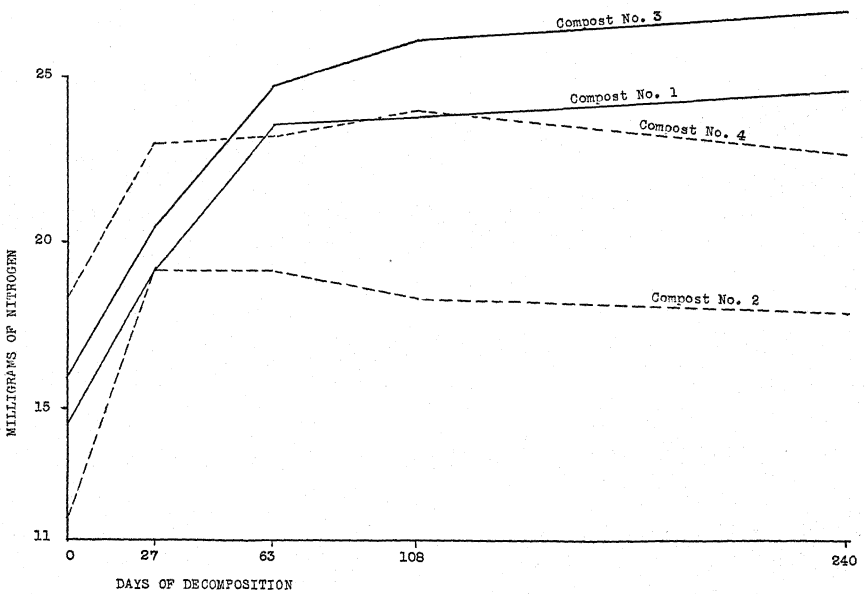
When the mushrooms ceased growing, the spent manure was analyzed. This took place 180 days after the composts were placed in the beds. Only the manure free from the surface soil was used for the analysis. The results of this analysis are given in table 10.

As a result of the growth of the mushrooms, the ash and total nitrogen content of the compost increased; a large part of the nitrogen has become water-soluble, while the ammonia was found to make up 20 to 35 percent of this water-soluble nitrogen. The cellulose has diminished only to a limited extent; however, the lignin concentration has diminished to a marked extent. During the process of decomposition of the manure in the compost, the relative concentration of the lignin has increased in proportion to the increase of the nitrogen and ash, showing that the lignin gradually accumulated and was not decomposed by the microörganisms of the manure. However, during the growth of the *Agaricus campestris*, the proportion of lignin to the total material diminished.

In the case of compost No. 1, the relative concentration of the ash increased from 15.49 to 23.2 percent between the time the material was placed in the beds and the end of mushroom growth, or in a ratio of 2 to 3; this points to a reduction in the bulk of the compost to two-thirds of the original material, on a dry weight basis. The lignin has decreased, in the same period of time, from 27.02 percent to 17.12 per cent. If the lignin were not decomposed by the mushroom fungus, it should have increased to 40 percent or so of the total material; the fact that it made up only 17.12 percent of the residual material shows that over a half of it was decomposed. In compost No. 2, the ash increased, in the same period of time, from 11.79 percent to 24.8 percent and the lignin decreased from 25.31 to 16.84 percent; similar calculations justify the conclusion that nearly two-thirds of the lignin originally present in this compost has disappeared as a result of the growth of the mushroom. In compost No. 3, the ash increased, in the same period, from 22.87 to 36.0 percent, and the lignin decreased from 26.69 to 12.95 percent, again accounting for a loss of more than two-thirds of the lignin. In compost No. 4, the ash increased from 32.91 to 50.3 percent, and the relative lignin concentration decreased from 26.10 to 18.09 percent, also showing a loss of more than a half of the total lignin content.

It is not as easy to interpret the changes in the protein content of the composts, as a result of the growth of the mushroom, since the methods of analysis do not permit the differentiation between proteins of plant and microbial origin. However, the decided increase of the total water-soluble

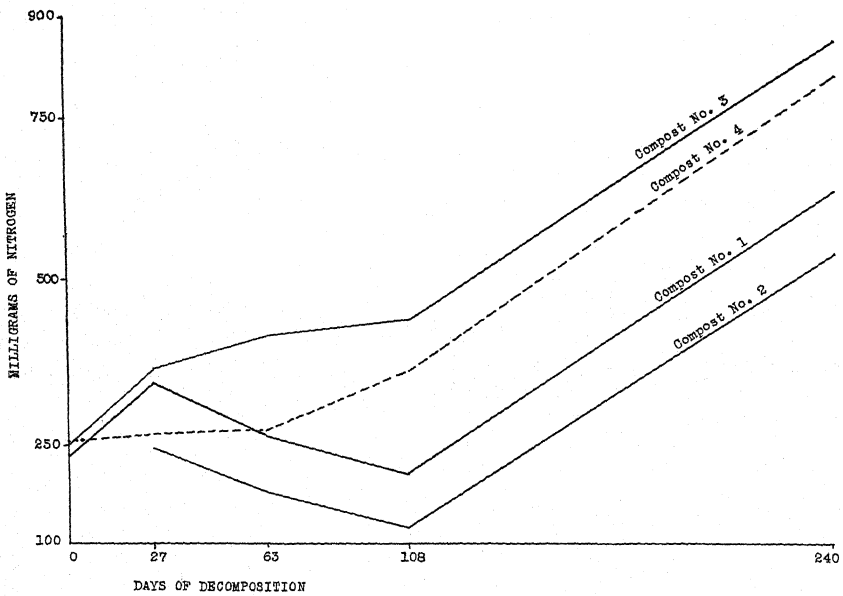
nitrogen, a considerable part of which is in the form of ammonia, shows that marked changes have taken place in the nature and concentration of the proteins in the composts. The results show very decidedly that the processes of decomposition of the manure compost by the mushroom fungus are quite different in nature from those changes which are brought about by microörganisms in the fresh manure, during its composting.



TEXT FIG. 2. Changes in relative concentration of nitrogen in the composts, during the process of decomposition. Milligrams of total nitrogen found in 1 gm. of dry material.

The difference in the attack of the constituents of the composts by the heterogeneous microbial population in the manure and by the mushroom fungus is well brought out in text figures 2 and 3, where the changes in total nitrogen and water soluble nitrogen in the different composts are recorded. The results show clearly that during the composting of the manure, there was a rapid rise in the total nitrogen content and an inappreciable change in the water-soluble nitrogen, while during the growth of the mushroom, the change in total nitrogen was inappreciable while the concentration of water-soluble nitrogen increased rapidly. These phenomena can be readily explained by the fact that during the process of composting, the fungi and bacteria of the manure decomposed primarily the cellulose and hemicelluloses; this led to the building up of considerable microbial cell substance, as a result of which all the nitrogen liberated as ammonia, in the decomposition of the organic nitrogenous complexes of the manure, was reconsumed and synthesized as microbial protein. During the growth of the *Agaricus*, the protein, both of the plant residues and of the microbial

cells, was readily attacked and the nitrogen made water soluble either directly or through the synthesis of mushroom mycelium.



TEXT FIG. 3. Transformation of nitrogen during the decomposition of the manure in the composts. Milligrams of water-soluble nitrogen in 100 gm. of dry material.

DECOMPOSITION OF MANURE FROM VARIOUS COMPOSTS BY A PURE CULTURE OF AGARICUS CAMPESTRIS

In view of the fact that during the growth of *Agaricus campestris* in the compost beds, the mushroom was always accompanied by various fungi and bacteria which were either originally present in the manure or came from the dust, it was frequently difficult to determine whether certain organic complexes, especially the cellulose and hemicelluloses, were decomposed by the *Agaricus* or by the accompanying fungi and bacteria. A careful study of this phenomenon could be undertaken only by the use of pure cultures of *Agaricus campestris* grown under sterile conditions. The four composts described above were used for this purpose. Known quantities of the moist material were placed in milk bottles; these were plugged with cotton and sterilized at 15 pounds pressure for 3 hours. Some of the bottles were inoculated with pure culture spawn of *Agaricus* and others were left uninoculated, as controls. After 104 and 256 days incubation, the contents of the uninoculated and inoculated bottles were analyzed. The results are reported in table 11.

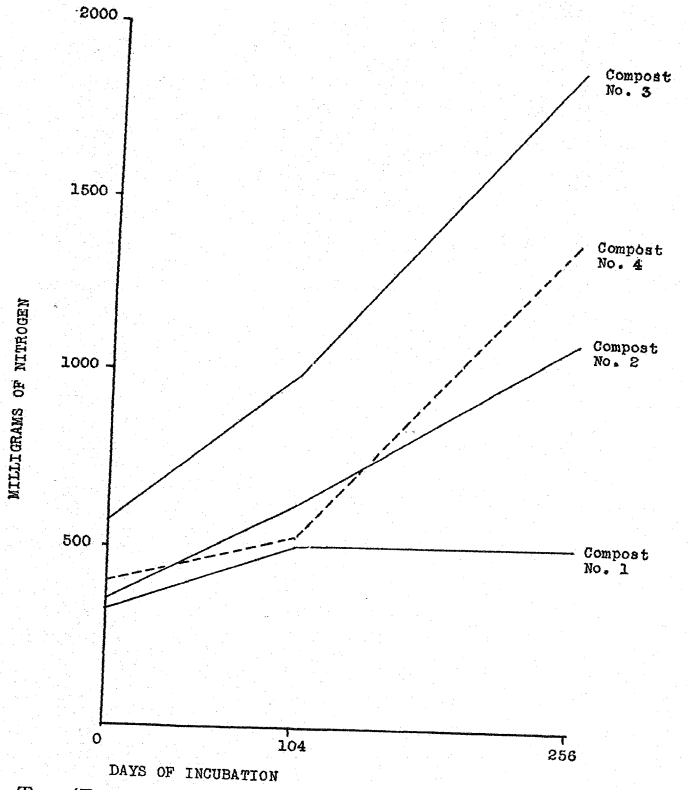
These results establish quite definitely that, although the mushroom fungus can attack cellulose and probably does so under certain conditions,

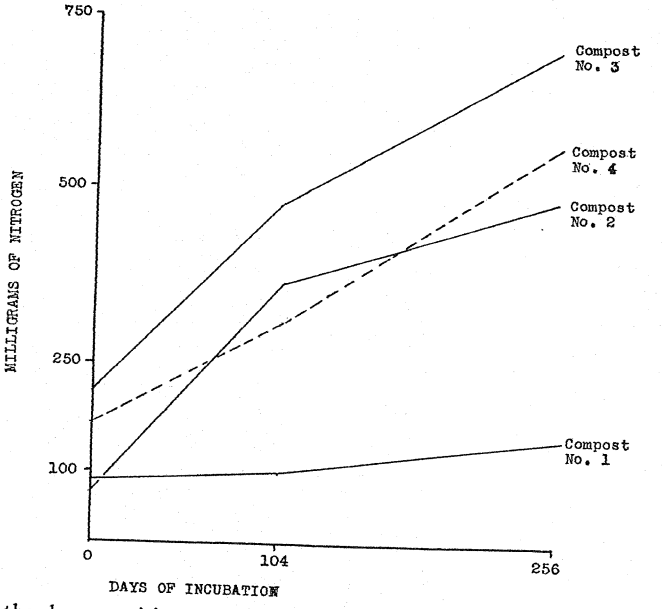
this carbohydrate forms only a small part of the organic constituents of the compost which it uses for its nutrition. The mushroom fungus feeds primarily upon the lignins, the proteins, and the hemicelluloses. In the case of the material from compost No. 1, the lignin has been reduced from 22.08 to 14.76 per 100 parts of the original material, a loss of over 33 percent of the lignin; in compost No. 2, the lignin was reduced from 23.69 to 5.33 parts per 100 of original material, a loss of 77.5 percent of the lignin; in compost No. 3, the lignin was reduced from 23.24 to 5.48, a loss of 76.5 percent of the lignin; in compost No. 4, the lignin was reduced from 21.08 to 4.75, a loss of 77.5 percent.

In addition to the lignins, the proteins have also undergone considerable decomposition. This is brought out by the marked increase in the water-soluble nitrogen. In the case of the material from compost No. 1, 20.8 percent of the nitrogen was in a water-soluble form at the end of the incubation period; in compost No. 2, 34.9 percent; in compost No. 3, 55.4 percent; and in compost No. 4, 47.3 percent of the total nitrogen was rendered water-soluble. In view of the fact that the mycelium of the fungus *Agaricus campestris* contains 40 to 50 percent of its nitrogen in a water-soluble form, we may conclude that a large part of the nitrogen left in the bottles was in the form of the mycelium of the mushroom. The hemicelluloses were also attacked by the *Agaricus*. The marked increase in cold-water-soluble organic matter is entirely at the expense of the development of the fungus mycelium. On comparing the increase of cold-water-soluble organic matter over the controls, one may again feel safe in concluding that the fungus mycelium makes up at least about a third of the total residual organic matter, since about 45 percent of the dry weight of the fungus mycelium is soluble in cold water (13).

Text figures 4 and 5 illustrate further the changes in water-soluble nitrogen and ammonia nitrogen in the material from the four composts by pure cultures of *Agaricus campestris*. The compost prepared from pure droppings liberated the greatest amount of ammonia and contained most water-soluble nitrogen, which checks up with the results obtained for this compost in the beds. Compost No. 1 did not allow as good a growth of the *Agaricus*, as one could judge from the comparatively limited decomposition of the lignin in this compost; as a result of this, there was a low accumulation of water-soluble and ammonia nitrogen.

It is of interest to report here another experiment, which may throw some light upon the influence of foreign organisms upon the growth of the cultivated mushroom. It is a common observation among mushroom growers that when the beds become infected with the fungus *Chaetomium*, the yields of mushrooms are markedly reduced. An opportunity to make certain observations upon this phenomenon occurred by a mere accident. Some of the material from compost No. 4 described above was placed in a series of 1-quart milk bottles; these were plugged with cotton, sterilized at





TEXT FIGS. 4 AND 5. FIG. 4. Formation of water-soluble nitrogen in the decomposition of various sterilized composts by pure cultures of *Agaricus campestris*. Milligrams of water-soluble nitrogen in 100 gm. of compost, on a dry basis. FIG. 5. Liberation of ammonia in the decomposition of various sterile composts by pure cultures of *Agaricus campestris*. Milligrams of ammonia nitrogen in 100 gm. of compost, on a dry basis.

15 pounds pressure for 2 hours, and inoculated with different spawn preparations. One bottle became infected with *Chaetomium* soon after the *Agaricus* began to develop, so that the lower part of the bottle had a fair growth of *Agaricus* and the upper part an abundant development of *Chaetomium*. At the end of 50 days incubation, the experiment was discontinued. The contaminated bottle was broken and the contents carefully separated into two portions, one containing largely the growth of *Agaricus* and the other that of *Chaetomium*. The primary purpose of this experiment was to compare the rapidity and nature of decomposition of the manure compost by two types of spawn, one Austrian and one American. The results of the decomposition of the compost by the two types of spawn, as well as the relative decomposition of the compost by *Agaricus*, as compared with *Chaetomium*, are given in table 12.

TABLE 12. *Decomposition of Composted Manure by Various Strains of Agaricus campestris and by Chaetomium sp.*

Nature of Culture	Control	American Spawn	Austrian Spawn	Contaminated Bottle	
				Agaricus	*Chaetomium*
		pure	pure	growth*	growth
Total dry weight, gm.	200.1	178.8	181.1	79.4	93.3
Ether-soluble portion, percent	0.85	0.31	0.35	0.67	0.53
Water-soluble organic matter, percent	5.71	14.67	11.70	10.68	7.23
Water-soluble nitrogen, percent	0.39	0.90	0.78	0.65	0.42
Hemicelluloses, percent	11.95	12.00	10.50	9.59	10.02
Cellulose, percent	14.40	14.62	13.40	8.82	7.09
Lignin, percent	22.87	14.63	15.26	18.59	20.97
Total nitrogen, percent	2.25	2.63	2.59	2.77	2.69
Ash, percent	32.6	33.6	33.4	34.8	35.6

* American spawn.

These results show that, although the American spawn was somewhat more active in decomposing the compost than the Austrian spawn, the two behaved about alike; this was shown by the extent of chemical transformations previously established for the growth of *Agaricus* upon a compost; namely, 1, an active decomposition of the lignins; 2, an active decomposition of the proteins and formation of a considerable amount of water-soluble nitrogenous compounds; 3, a limited decomposition of the cellulose; 4, the formation of considerable water-soluble organic matter. The compost infected with *Chaetomium* behaved quite differently; there was a marked reduction in the cellulose, a limited reduction of the lignin (even that may be due to a partial development of the *Agaricus* mycelium) and a small increase in the water-soluble nitrogen and water-soluble organic matter. The other part of the bottle containing the *Agaricus* behaved similar to the regular *Agaricus* cultures, although to a less extent, as shown by a smaller decomposition of the lignin, production of less water-soluble organic matter and nitrogen and greater cellulose decomposition (the latter possibly due to presence of some *Chaetomium* in the material). These results show

that the injurious effect of *Chaetomium* upon *Agaricus* cannot be due to competition for available food, but is probably a result of direct injury, although further work in this connection is desirable.

Decomposition of Fresh and Composted Manure by Pure Cultures of Agaricus campestris

The investigations just reported on the decomposition of manure by *Agaricus campestris* were carried out with composted manure, in which numerous changes have been brought about by the previous growth of fungi and bacteria, chief among which is a reduction of cellulose and hemicellulose content and an increase in lignin and protein content. In order to compare the decomposition of the various organic chemical complexes by the mushroom fungus, as well as to throw further light upon the nutrition of this organism, it was found desirable to compare its action upon fresh horse manure and upon composted horse manure, both of which have previously been washed to remove the water-soluble substances. A series of bottles containing definite quantities of the two types of manure were prepared, plugged, sterilized as usual, and inoculated with spawn prepared from two strains of *Agaricus campestris*. The bottles were placed in a constant temperature thermostat (27° C.) and incubated; after different intervals of time some of the bottles were analyzed. The contents were first removed, weighed, and the moisture content determined. Some of the analyses, as ammonia and total nitrogen, were made on the fresh material, while for the complete proximate analysis, the culture material was first air dried. The results of the analyses are reported in tables 13 and 14.

These results are in full agreement with those reported previously. The growth of the cultivated mushroom upon composted manure brought about a marked increase in the water-soluble material, a considerable reduction in the lignin content, a transformation of the water-insoluble protein into water-soluble forms of nitrogen, an appreciable reduction in the hemicelluloses and a small reduction in the cellulose content. In the original compost, the nitrogen present in the form of ammonia made up only a small fraction of the total nitrogen (2.63–2.65 percent); however, at the end of the growth period of the mushroom, the ammonia nitrogen was found to form 16 to 26 percent of the total nitrogen. It is interesting to note that the lignin-nitrogen, or that part of the nitrogen which is found in the lignin fraction (determined as acid lignin) diminishes proportionally with the decrease in the total lignin content; this would tend to indicate that either the lignin and protein are decomposed alike, or, which is more likely, that the lignin and the protein are present in the compost in the form of a complex, the protein of which cannot be attacked, unless the lignin is acted upon as well. That the latter assumption is probably the correct one is shown elsewhere (12).

TABLE 13. *Decomposition of Composted Horse Manure by Agaricus campestris. Results Reported on Percent Basis of Dry Material*

Nature of Culture	Period of Incubation, Days	Total Weight*	Ether-soluble Portion	Water-soluble Organic Matter	Hemicelluloses	Cellulose	Lignin	Ash	Total Nitrogen	Water-soluble Nitrogen	NH_3-N	Percent of NH_3-N / total N	Percent of Lignin-N / total N
Control	54	100	1.12	3.42	7.65	14.53	24.43	45.2	1.508	0.230	0.040	2.65	—
Brown strain of *Agaricus*	54	87.4	0.54	9.50	6.84	13.43	11.90	46.1	1.584	0.660	0.075	4.73	—
White strain of *Agaricus*	54	93.7	0.61	9.65	7.02	15.07	15.08	45.5	1.615	0.601	0.091	5.62	—
Control	215	100	1.26	3.24	6.80	12.51	22.91	45.2	1.560	0.142	0.041	2.63	42.3
Brown strain of *Agaricus*	215	82.4	0.48	18.70	2.64	7.51	7.77	56.9	1.770	0.884	0.283	16.00	14.1
White strain of *Agaricus*	215	85.0	0.43	25.05	2.02	6.66	6.31	61.5	1.790	1.172	0.465	25.98	12.1

* Calculated to 100 gm. of original material in control bottles.

TABLE 14. *Decomposition of Fresh Horse Manure by Agaricus campestris. Results Reported on Percent Basis of Dry Material*

Nature of Culture	Period of Incubation, Days	Total Weight*	Ether-soluble Portion	Water-soluble Organic Matter	Hemicelluloses	Cellulose	Lignin	Ash	Total Nitrogen	Water-soluble Nitrogen	NH_3-N	Percent of NH_3-N / total N	Percent of Lignin-N / total N
Control	77	100	2.30	5.60	18.6	20.7	20.0	16.9	1.44	—	—	—	—
Brown strain of *Agaricus*	77	94	1.40	11.90	14.4	20.8	15.7	18.7	1.65	—	—	—	—
Control	237	100	2.46	6.31	18.1	21.4	21.3	16.9	1.45	0.32	0.039	4.7	46.7
Brown strain of *Agaricus*	237	63	1.08	34.22	5.5	9.7	11.0	29.1	2.71	1.08	0.318	11.7	11.0

* Calculated to 100 gm. of original material in control flasks.

Although the changes in the organic constituents of the manure are very considerable, as shown by the reduction of the three major groups, namely the lignins, hemicelluloses, and cellulose, the actual reduction in the total weight of the compost is relatively small. This indicates that a large proportion of the residual material consists of synthesized mycelium of the *Agaricus*. That this is the case is brought out by the very considerable amount of water-soluble nitrogen. After 215 days of decomposition, the relative amount of water soluble to total nitrogen has increased from 9 percent to 50 and 65 percent for the two strains, respectively. The fact that the mushroom mycelium consists of 40 to 50 percent of water-soluble material and the fact that a large part of the water-soluble substances in residual material is probably derived directly from this mycelium, would tend to confirm the above assumption.

The decomposition of the washed and sterilized fresh manure by a pure culture of *Agaricus campestris* tends to bring further light upon the problem of mushroom nutrition. The organism grew well upon this material, and brought about transformations similar to those found in the case of the composted manure, the only difference being that somewhat less of the lignin was decomposed and somewhat more of the cellulose. Here, as well, the marked increase in water-soluble organic matter and in water-soluble nitrogen tends to indicate that a large part of the residual material consists of synthesized fungus mycelium.

Discussion

The growth of *Agaricus campestris* upon a compost of horse manure is characterized primarily by the decomposition of the lignins and of the proteins and by an increase in the water-soluble organic matter and the water-soluble nitrogen. Although the hemicelluloses and cellulose are also attacked by the fungus, and frequently to quite a considerable extent, which points to the ability of *Agaricus* to attack and utilize these plant constituents, still this is not as characteristic of the growth of the organism and does not differentiate its action from that of the microbial population of the manure.

In the process of composting of manure, there is a marked reduction in water-soluble material, a considerable destruction of the hemicelluloses and cellulose, and an increase in the relative proportion of the lignin and of the organic nitrogenous complexes. The latter are built up at the expense of the ammonia nitrogen in the manure, whereby the cellulose and hemicelluloses are used as sources of energy and microbial cell substance is synthesized. The accumulation of the lignin and the synthesis of microbial cell substance are thus found to be two phenomena resulting from the decomposition of manure in the process of composting, which are of decided benefit to the growth of the cultivated mushroom, by supplying it with the nutrients most essential for its development.

In the preliminary composting of manure there is a reduction of the readily decomposable substances by the heterogeneous bacterial and fungus flora of the manure; this results in the evolution of considerable heat which would injure the growth of the mushroom; this is another important result of composting of the manure to prepare it for the growth of the mushroom. By composting manure for a period of 4 to 8 weeks, turning it occasionally so as to favor aerobic processes of decomposition, the manure compost gradually becomes a poor medium for the growth of the common fungi and bacteria inhabiting the manure. The mushroom fungus grows upon these complexes which are not decomposed readily by the bacteria and fungi of the manure, namely the lignins and the hemicelluloses; or which are synthesized in the process of decomposition of the manure, namely the organic nitrogenous complexes. As a result of these phenomena, the process of composting of manure is of considerable benefit for the growth of the cultivated mushroom, *Agaricus campestris*.

Summary

1. A study has been made of the chemical composition of composts of horse manure prepared for the growth of the cultivated mushroom, during the process of composting and during the growth of the mushroom, and of the spent manure.

2. As a result of the composting of manure, there is a reduction in the water-soluble substances, in ammonia nitrogen, in hemicelluloses, and in cellulose, accompanied by an increase in the lignins, total nitrogen, and ash.

3. The growth of the cultivated mushroom upon the compost is marked by a decided decrease in the lignin content and an increase in the water-soluble organic matter, including water-soluble organic nitrogen compounds and ammonia. These results point to the ability of the mushroom fungus to use as nutrients the lignins and proteins of the compost.

4. Special composts were prepared of two types of horse manure, with and without bedding, and with and without additional straw. These composts were kept under controlled conditions and careful records made of changes in temperature, total weight, and moisture. At the end of the period of composting, the composts were placed in beds, which were spawned with *Agaricus campestris* and the mushrooms grown to maturity.

5. The yield of mushrooms in the beds was about 2 pounds per square foot, although the conditions in the beds were not most favorable for mushroom production, due to a lack of insect control, as well as a lack of humidity and temperature control. The largest yield of mushrooms was obtained from the beds containing the compost prepared from manure droppings, free from bedding; however, these mushrooms were small and hardly marketable. The best mushrooms and a fairly large yield were obtained from the compost prepared from the manure which included the bedding and which received, in addition, 25 percent of wheat straw, on a

dry weight basis, as well as the necessary amount of nitrogen and mineral nutrients.

6. In the process of composting of the manure, there was a rapid development of fungi and bacteria. These brought about a reduction in the water-soluble substances of the manure, in the hemicelluloses and to a less extent in the cellulose; these processes were accompanied by an increase in the lignin content, the total nitrogen content, and the ash. The last three complexes increased in proportion to the decomposition of the manure as a whole; the lignin in the manure had not decomposed during the process of composting; the insoluble nitrogen, chiefly in the form of protein, was largely synthesized by the cells of the microörganisms decomposing the manure.

7. Since *Agaricus campestris* is a non-chlorphyllous organism, it cannot use the CO_2 of the atmosphere, but must obtain its energy from the decomposition of the organic substances in the compost, and the nutrients necessary for the synthesis of its cell substance (including both its extensive mycelium and the edible mushroom) from the inorganic and organic constituents of the compost. Since the ash content of this fungus is only 13.3 percent (on a dry basis), the amounts of the organic substances which it has to decompose are very considerable.

8. The mushroom fungus does not use the compost as a whole for its nutrition and for its energy needs; it seems to attack certain organic complexes in preference to others. The complexes that the mushroom uses by preference are those which were accumulated in the process of composting of the manure, namely the lignins and the organic nitrogenous complexes, and to a less extent the hemicelluloses and the cellulose.

9. In the process of growth of the mushroom upon the compost, the lignins are markedly reduced, not only in absolute quantity but also relative to the other organic complexes, while the nitrogenous substances are transformed from an insoluble organic into soluble organic and mineralized (NH_3) forms. The high content of water-soluble nitrogen is a result of the fact that the mushroom mycelium is high in nitrogen (6.44 percent of its dry weight), with nearly a half of this nitrogen in a water-soluble form.

10. A large part of the compost left after the growth of the mushroom consists of the mycelium and products of decomposition of the fungus.

11. The above results were further confirmed by growing pure cultures of the mushroom upon sterile substrates of composted and fresh horse manure.

The authors are indebted to the Mushroom Growers Association of Pennsylvania for supporting this investigation; to Dr. J. F. Styer of E. H. Jacob Co. and to Mr. Rettew of the West Chester County Laboratories for supplying composts, spawn and other necessary material and for suggestions and advice fully given; and to Mr. E. R. Purvis of this laboratory for assistance in some of the analytical work.

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THE ANATOMY OF THE CASTOR BEAN AS CONDITIONED BY LIGHT INTENSITY AND SOIL MOISTURE

WM. T. PENFOUND

(Received for publication January 17, 1932)

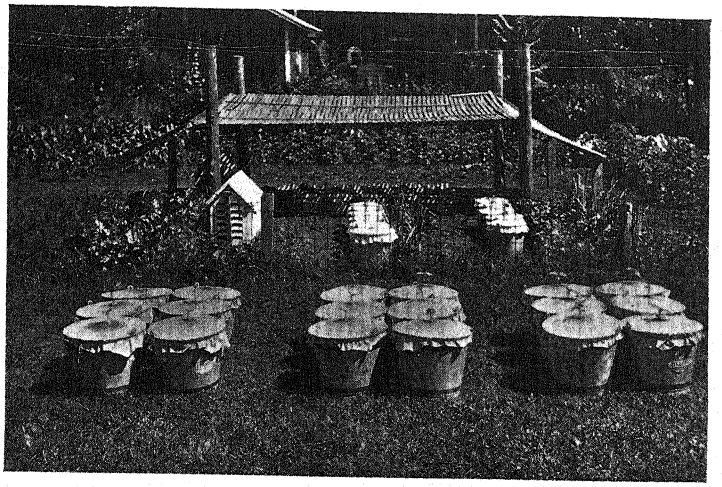
The effect of physical factors on the anatomy of plants has recently become the object of considerable investigation. Most of these researches have dealt with the effect of various physical factors on the structure of leaves. In the present investigation an attempt has been made to determine the effects of various combinations of light intensity and soil moisture on the structure of the roots, stems, and leaves of the castor bean plant.

METHODS

The experiment was conducted in a garden at Urbana, Illinois, in the summer of 1927. Castor bean plants were grown in four combinations of light intensity and soil moisture, viz: full sunlight–wet soil, full sunlight–dry soil, shade–wet soil, and shade–dry soil. Seedlings of similar vigor and size of cotyledons were transplanted to galvanized buckets of fourteen-liter capacity containing thoroughly mixed and sieved silt loam. The soil in twelve of the containers had an available water content (as based on the dry weight of the soil) of 21 percent and in twelve others the available water was only 6 percent. The soil surfaces were covered with a heavy oilcloth seal and the plants were allowed to develop without additional water. A variation in light intensity, without appreciably affecting the air temperature or relative humidity, was obtained by means of a specially constructed lath shelter, the soil underneath being covered with black cinders (text figs. 1 and 2). The light intensity, as calculated from an average of hourly readings from 7 A.M. to 6 P.M., and based on mid-day sun on June 22, was 90 percent in the full sunlight and 18 percent under the shelter.

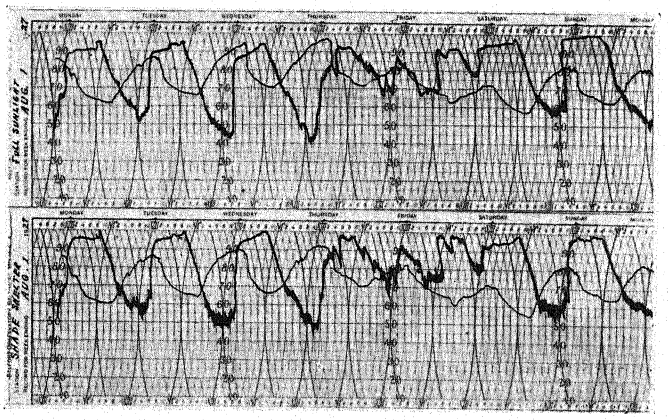
One set of six plants growing in wet soil was placed in full sunlight and a similar one was deposited under the shelter. A similar disposition was made of those growing in dry soil. Under these conditions growth proceeded for a period of 24 days, from July 18 to August 12. During this time the air temperature was comparatively low and the relative humidity was moderately high (text fig. 3). Although relatively humid conditions prevailed during the growth period the plants grown in dry soil and full sunlight experienced wilting on certain days between August 1 and August 12. In addition the plants growing in dry soil under the shelter experienced moderate wilting on two different occasions. Nevertheless the available water content at the end of the growth period was well above the wilting

coefficient in both the upper and lower five inches of soil in all the containers. This demonstrates that mesophytes, such as the castor bean, need a considerable margin of available water in order to thrive in an atmosphere of relatively high evaporating power.



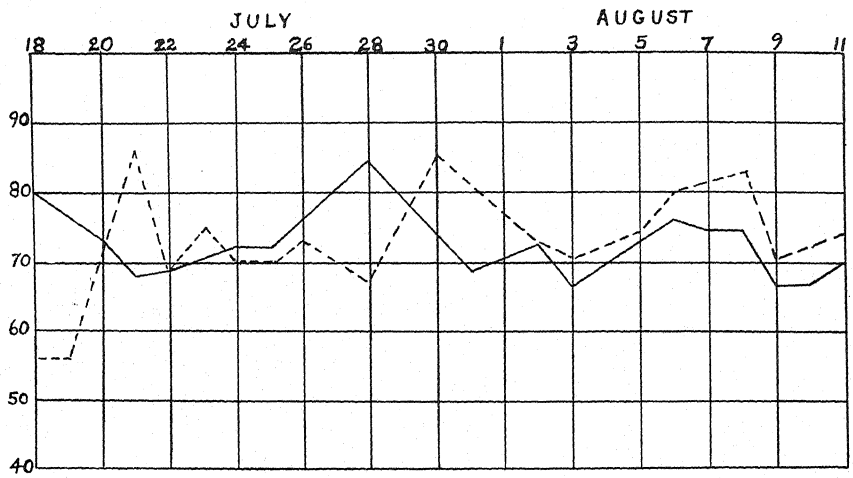
TEXT FIG. 1. Shelter, hygrothermographs, and batteries of plants.

The average transpiration per plant per day varied directly with the degree of insolation and water content of the soil. Although the transpiration per unit area in full sunlight was double that in shade, there was little difference in the transpiration rate in the two conditions of soil moisture (table 3). This result is not in accord with previous findings on the same subject. Whether this difference is due to the species used or to the con-



TEXT FIG. 2. Hygrothermograph records in full sunlight (above) and under the shelter (below) for the week ending August 1, 1927. Light lines, temperature; heavy lines, humidity.

ditions of the experiment is not certain. But it is possible that future experiments will show that species differ considerably in their transpiration rates in soils of diverse water content.



TEXT FIG. 3. Average daily temperature (solid line) and average daily relative humidity (dotted line) during growth period.

RESULTS

External Features

The development of the plants at the end of the growth period varied directly with the water content of the soil and in the main with the degree of insolation (table 1), but the height of the plants was similar in both conditions of insolation and the size of cotyledons and leaves was greater under the shelter. Despite these facts the root systems of the plants in full sunlight were more extensive.

TABLE 1. *External Features of the Castor Bean Plant Grown in Varied Combinations of Light Intensity and Soil Moisture. All Measurements in Millimeters*

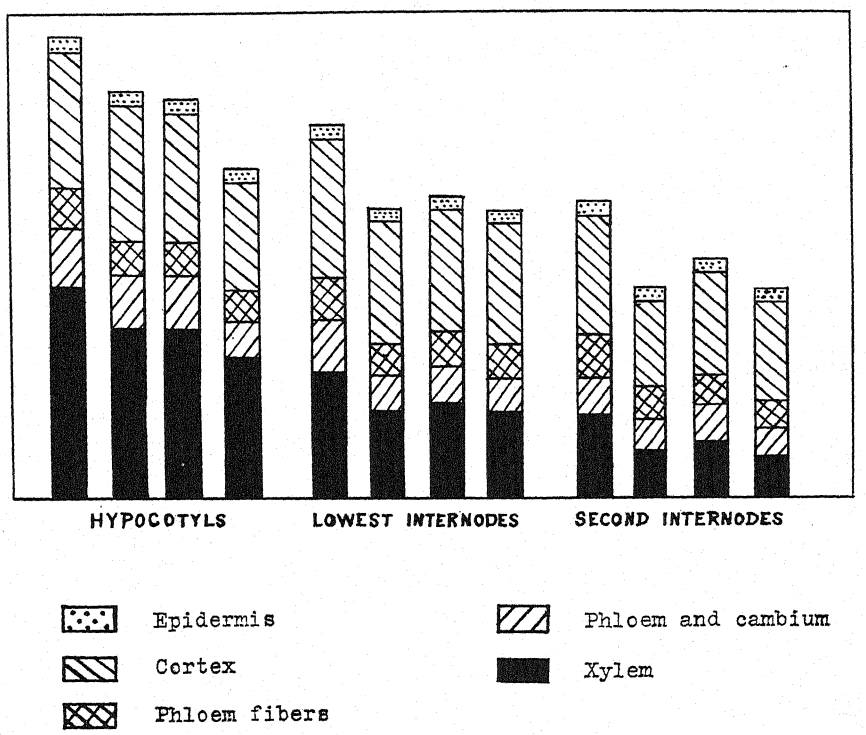
	Sun–wet	Sun–dry	Shade–wet	Shade–dry
Av. diam. of roots	0.8	0.6	0.8	0.7
Av. max. length roots	355.0	304.0	254.0	203.0
Av. diam. of stems	3.6	2.7	2.9	2.6
Av. height of stems	53.0	43.5	54.5	43.5
Width of cotyledons	39.0	33.0	43.0	39.0
Length of cotyledons	51.0	42.0	58.0	49.0
Width of lowest leaves	59.0	45.0	75.0	52.0
Length of lowest leaves	66.0	48.0	83.0	59.0

Internal Features

A comparison of the structure of roots grown under varied habitat conditions is rather hazardous since one can never know the exact age of

the roots with which he is dealing. Prepared slides were made from portions of the main lateral roots one-third the distance from their bases to their tips. A study of these sections showed, in general, that the thickness of the roots and the amount of xylem varied with the degree of insolation and the amount of soil moisture.

The data on the structure of the stems indicate that the castor bean is somewhat less modified by varied conditions of light intensity and soil moisture than the sunflower. In general the plants were much better developed in the combination of full sun and wet soil, and varied only slightly in the other three light intensity–soil moisture combinations (text fig. 4). In both the hypocotyls and the internodes the size of the cells



TEXT FIG. 4. Graph showing the effect of light intensity and soil moisture on the stem structure of the castor bean. Left to right: full sunlight–wet soil; full sunlight–dry soil; shade–wet soil; shade–dry soil.

varied in the same direction as the amount of soil moisture and to a less extent with the degree of insolation. Although the wall thickness of the mechanical elements varied in the same direction as the amount of soil moisture and the degree of insolation it was influenced more by the latter (table 2).

TABLE 2. *The Relation of Light Intensity and Soil Moisture to the Size and Wall Thickness of the Cells in the Stems of the Castor Bean. All Measurements in Microns*

	Sun–wet	Sun–dry	Shade–wet	Shade–dry
	Hypocotyls			
Wall thickness of bast fibers	5.6	5.4	2.8	1.7
Wall thickness of wood fibers	2.1	2.0	1.7	1.4
Diameter of vessels	35.0	32.0	31.0	28.0
Diameter of pith cells	94.0	88.0	92.0	78.0
	Lowest Internodes			
Wall thickness of bast fibers	2.1	2.0	1.7	1.6
Wall thickness of wood fibers	1.7	1.6	1.6	1.4
Diameter of vessels	30.0	24.0	28.0	22.0
Diameter of pith cells	81.0	64.0	68.0	58.0

In all the stem-sections the actual xylem and vascular areas, as well as the percentage xylem and vascular areas, varied in the same manner (table 3). They were highest in the full sunlight–wet soil set and progressively lower in the shade–wet soil, full sunlight–dry soil, and shade–dry soil combinations. It appears, therefore, that soil moisture plays a greater role in vascular development than the degree of insolation. Since the development of vascular tissue parallels closely the average total transpiration per plant per day it follows that the degree of vascular development and the amount of water loss are closely interdependent (table 3).

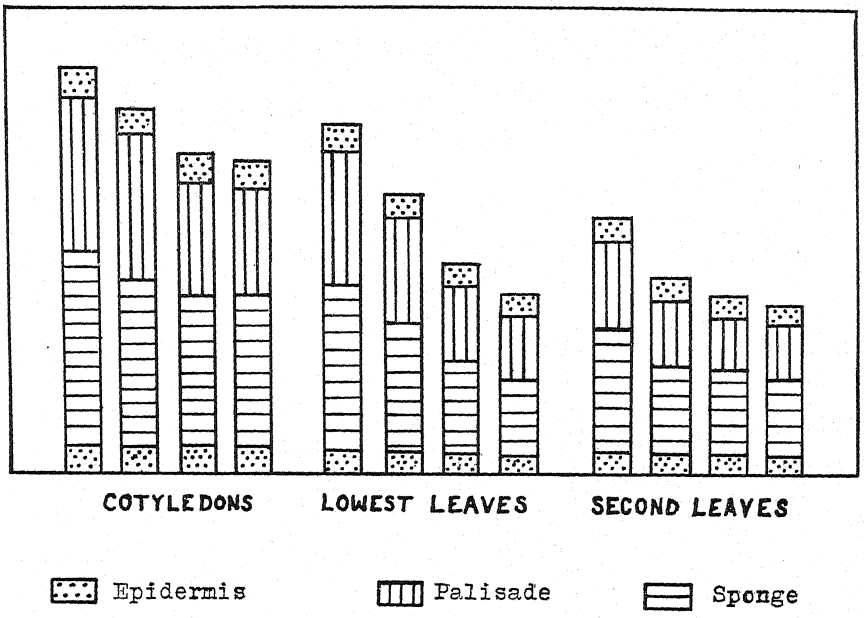
TABLE 3. *The Relation of Light Intensity and Soil Moisture Content to the Conducting and Transpiring Systems of the Castor Bean*

	Sun–wet	Sun–dry	Shade–wet	Shade–dry
Xylem area (sq. mm.)	1.84	0.89	0.97	0.55
Vascular area (sq. mm.)	0.39	0.24	0.26	0.17
Percent xylem	16.1	11.8	12.6	8.4
Percent vascular tissue	3.6	3.2	3.4	2.6
Av. leaf area plant (sq. cm.)	193.0	91.0	280.0	132.0
Transpiration (cc. per day): Av. per plant	18.2	7.6	11.4	4.9
Transpiration (cc. per day): Av. per sq. dm.	9.4	8.4	4.1	3.7
Vascular area of hypocotyls / Av. leaf area per plant	0.20%	0.26%	0.09%	0.13%
Av. transp. per plant per day / Vascular area of hypocotyls	46.5	31.5	44.0	29.0
Av. transp. per sq. dm. per day / Vascular area of hypocotyls	24.0	35.0	16.0	22.0

In both the cotyledons and leaves the development of the mesophyll and midvein showed considerable differences in their structure in the various light-moisture conditions. The stomata were more numerous per unit area in the full sunlight–dry soil set and were progressively less abun-

dant in the full sun–wet soil, shade–dry soil, and shade–wet soil combinations. They were largest, however, in the full sun–wet soil combination and similar in size for the other three sets. These results showed that light intensity was more important in its effect on the number of stomata than soil moisture. This is due to the fact that sunlight initiates the formation of additional stomata whereas soil moisture affects the number of stomata per unit area only by affecting the size of all the epidermal cells.

The development of the mesophyll was better in full sunlight than in shade and slightly greater in wet soil than in dry soil (text fig. 5). Con-



TEXT FIG. 5. Graph showing the effects of light intensity and soil moisture on the structure of the mesophyll of the castor bean plant. Left to right: full sunlight–wet soil; full sunlight–dry soil; shade–wet soil; shade–dry soil.

spicuous differences obtained between the leaves of wet soil and dry soil plants in full sunlight but the differences were slight under the shelter. It should be noted, also, that the percentage depth of palisade in the leaves from full sun plants was not much greater than in the shade plants. These results suggest that the mesophyll of the castor bean leaf is not very plastic and probably is not as responsive to changes in physical factors as the hypocotyls and internodes.

In the midvein the development was best in the full sun–wet soil combination, about equal in the full sun–dry soil and shade–wet soil sets, and poorest in the shade–dry soil combination. This applied to the depth of the epidermis, collenchyma, chlorenchyma, parenchyma, phloem, and

for the size of the xylem masses. Nevertheless both the percentage xylem and vascular areas were similar in all four combinations of light intensity and soil moisture.

Relation of Varied Light Intensity and Soil Moisture to the Water Balance

When plants are grown in conditions favoring a rapid water loss the interrelation of the area of the root system, the conducting area of the stem, and the transpiring area are of critical importance. Since the data on the root systems are incomplete no quantitative results are possible. But the root-top ratio was much greater in full sunlight than under the shelter. In both the hypocotyls and lowest internodes the ratio of either the xylem or vascular areas to the leaf area was much higher in full sunlight than under the shelter, but there was little difference in these ratios between wet soil and dry soil plants either in the fully exposed or sheltered condition (table 3).

Nevertheless the ratio of the average transpiration per plant per day either to the xylem area or the vascular area was higher in the wet soil than in the dry soil (table 3). There was, however, no significant difference in these ratios between the plants in full sunlight and under the shelter. This means that the rate of flow (amount of water passing over unit vascular area in a given period of time) varied directly with the soil moisture content but was similar in the two conditions of light intensity. On the other hand, the ratio of the intensity of transpiration to the vascular area varied directly with the degree of insolation and with the aridity of the soil. An examination of the data (table 3) shows that the average transpiration per plant per day varied in the same manner as the ratios of the transpiration per plant to the vascular area. This parallelism helps to explain the fact that the ratios of the transpiration per plant to the vascular area were relatively low in the dry soil plants.

Discussion

The results of Zalenski (Maximov, 1929) indicated that strong insolation and dry air were both effective in inducing xeromorphic structure. In regard to the effect of soil moisture Rippel (Maximov, 1929), Penfound (1931), and others have shown that many xeromorphic features were promoted by a low soil moisture content.

In the present experiments the root systems were much better developed in sunlight despite the fact that the leaf area was greater in the shaded situation. Because of this the absorbing system was relatively more efficient and the transpiration per plant greater in the condition of maximum insolation. In general the wet soil and dry soil plants differed more from each other in full sunlight than under the shelter. This was due to the fact that in full sunlight the water content of the dry soil plants was lowered to an unfavorable degree in periods of high evaporational stress.

The development of the mechanical system varied in the same direction as the light intensity and the soil moisture content. It appears, however, that the thickness of the walls is dependent more upon light intensity than on soil moisture, probably due to the need of abundant carbohydrates for the production of wall substance. Since the percentage of mechanical tissue in the stem to the leaf area was much greater in sunlight than under the shelter it is evident that the plants in full sunlight have a more efficient mechanical system than those under the shelter. Cannon (1905), and Rippel (Maximov, 1929) found a better development of vascular tissue in dry soil, but I have found a much better vascular development in soil of high water content, especially if combined with maximum insolation. From this investigation, and others on the same subject, it appears that conditions which favor a rapid water absorption and water loss are also efficient in promoting the development of vascular tissue.

Rübel (1920) studied the effect of varied light intensity on the anatomy of the sunflower and found that the ratio of the vascular area of the stem to the transpiring surface was nearly a constant, but the results of my experiments have shown a consistently higher ratio in the sun plants. Nevertheless the amount of water passing over unit vascular area in a given period of time was similar in both conditions of insolation. In Rübel's experiments the ratio of the intensity of transpiration to the vascular area was higher in the shade plants whereas in the present experiments the ratio was lower in the shade plants.

The ratio of the vascular area to the leaf area was similar in both conditions of soil moisture. Nevertheless the rate of flow was higher in the wet soil than in the dry soil. This is not in agreement with the results on the sunflower (Penfound, 1931). It should be noted, however, that the transpiration rate for the castor bean was greater in the wet soil whereas the rate of water loss in the sunflower varied with soil aridity. The above results suggest that species of plants may vary in their reactions to physical factors, and that the results obtained in one experiment may not obtain in another especially if one is dealing with different species, plants of different ages, or with different habitat conditions.

Summary

In studies on castor bean plants grown in varied combinations of light intensity and soil moisture the following conclusions were reached:

1. In the roots the amount of xylem and the average total diameter varied in the same direction as the light intensity and the amount of soil moisture.

2. In the stems the cross-sectional, xylem, and vascular areas, the percentage xylem and percentage vascular areas as related to the cross-sectional areas, the size of the cells, and the wall thickness of the mechanical elements varied directly with the degree of insolation and the soil moisture content.

3. In the leaves the number of stomata per unit area varied directly with the degree of insolation and the aridity of the soil, and the development of the mesophyll, as well as that of the midvein, was slightly better in conditions of maximum insolation and high soil moisture content.

4. The ratio of the vascular area of the stem to the leaf area was much higher in conditions of maximum insolation but varied little with the amount of soil moisture. Nevertheless the *ratio of the average transpiration per plant either to the xylem area or to the vascular area* was similar in both conditions of light intensity but varied in the same direction as the amount of soil moisture.

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INFLUENCE OF VISIBLE AND ULTRAVIOLET RAYS ON THE STABILITY OF PROTOPLASM

W. W. LEPESCHKIN

(Received for publication November 11, 1931)

It is well known that protoplasm subjected to the action of strong light is gradually desintegrated and dies. Protoplasm is especially sensitive to ultraviolet rays. At the same time, these rays can also produce a stimulating effect on growth, development, and cell division. Mitogenetic rays which presumably accelerate mitosis have also been shown to be ultraviolet in character. The cause of the destructive effect of light presumably lies in a chemical change of protoplasmic proteins, as shown by the work of Henri, Cleaves, Clark, Young, and others. The cause of the stimulating effect of ultraviolet rays is unknown. In the author's previous papers evidence was presented that the effect of light on protoplasm can be demonstrated even if no visible change in its structure or physiological response can be observed. The experiments made on *Spirogyra* showed that visible light (even diffuse light) decreases the stability of its protoplasm against high temperature and mechanical injuries. *Spirogyra* showed daily variations in the stability of its living matter, this stability being greater at night than by day.[1]

In the present paper experiments are described in which some details of the action of visible and ultraviolet rays on the resistance of plant protoplasm to poisons were investigated.

MATERIAL AND METHODS

Two kinds of cells were used, yeast cells, and leaf cells of *Elodea canadensis*. The poisons used were alcohol and iodine. In the case of yeast cells the method was similar to that used in the author's previous experiments on the protective effect of narcotics on protoplasm.[2]

The standard suspension of yeast in spring water (pH = 7.3, 0.0019

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[2] **Lepeschkin, W. W.** 1929. Amer. Jour. Bot. 26: 208–218.

[The Journal for June (19: 443–546) was issued July 1, 1932.]

percent Ca, 0.0022 percent Cl) contained three g. of Fleischmann's compressed yeast in 100 cc. This suspension was poured into test tubes (capacity five cc.) of Pyrex glass or quartz, one cc. of the suspension into each tube closed with a cork; then some of the tubes were wrapped in black sateen (three layers) and all tubes were immersed in a water bath of an approximately constant temperature and exposed to direct sunlight. After some period of time (indicated in every experiment) the tubes were taken from the water bath, and the resistance of the yeast cells to alcohol was determined. One cc. of a 46 percent solution of ethyl alcohol [3] in water was added to the yeast suspension in each tube. After exactly 10 minutes (measured with a stop-watch) one cc. of a 0.075 percent solution of methylene blue was added to the mixture in each tube, and the number of the colored cells in percent of the whole cell number was determined for every tube. The figures given below are averages obtained from at least six figures. The maximum possible error is about three percent of the whole number of dead cells in a single experiment. As the simultaneous action of the dye and alcohol brings about a slow killing of the cells, the counting of dead cells lasted not longer than five minutes beginning at the moment of the addition of the dye.

In some experiments alcohol was added before the exposure to sunlight to prevent multiplication and synthetic processes in the cells. The concentration of alcohol in the suspension was this time 17 or 18 percent.

The application of the method just described to *Elodea* showed that the counting of dead cells which had colored protoplasm, nucleus, and chloroplasts is not so exact as in the case of yeast cells because the dead cells are scattered unevenly among living cells, and the cells situated on the leaf tip and edges die more easily. Thus only the relative area occupied by dead cells of the lower cell layer could be measured by drawing it and the whole leaf area by means of a Leitz drawing eye-piece (direct method). The colorimetric method is more exact.

A special colorimetric scale was prepared. About 20 leaves cut from one branch of *Elodea* were immersed in a 12 percent alcohol solution. After a given time, single leaves were taken from alcohol in succession and put in a 0.005 percent solution of methylene blue to which 0.5 percent of NaCl was added to avoid adsorption. There they remained for exactly 10 minutes. Then the relative area of dead cells (expressed in percent) was determined, and the leaves were dried with blotting paper, fastened to slides in a successive series, and covered with slides. In a few hours the leaves dried completely and could be used for a comparison with leaves colored in experiments. The number of dead cells in percent in the leaves of the scale were the following:

Periods of time (minutes) during which the leaves remained in 12% alcohol	3	6	9	12	15	18	21	24	27	30	33	36	39
Number of dead cells (%)	2	5	8	12	16	20	30	40	50	60	70	80	85

[3] By percent is always understood grams in 100 cc. of the solution.

In the case of iodine as a poison the scale used was as follows:

Period of time (minutes) during which the leaves remained in in a 0.02% iodine[4]	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Number of dead cells (%)	3	5	10	12	15	18	20	25	30	40	45	55	60	85	95

If the color of a leaf used in an experiment and dried was between the colors of two neighboring leaves of the scale, the number of dead cells in this leaf was evidently between the numbers corresponding to the leaves of the scale. The numbers obtained should be corrected because light changes the permeability of protoplasm, and the living cells of *Elodea* absorb methylene blue. Special experiments made to estimate the possible influence of the permeability change showed that uninjured leaves absorb methylene blue from a 0.005 percent solution (to which 0.5 percent NaCl was added), to an amount corresponding to T = 8.2 of the scale used for the determination of the permeability.[5] When the leaves of the same *Elodea* branch had been immersed in 12 percent alcohol for 20 minutes the amount of the dye absorbed was T = 28. Since at the same time only 22 percent of dead cells were found, the amount of the dye absorbed by living cells was $\frac{8.2 \times 78}{100} = 6.4$; that absorbed by dead cells was $28 - 6.4 = 21.6$. Suppose now that after the action of light the permeability of protoplasm increased m times and that the true number of dead cells in a leaf, in percent, is x, while the number found by the colorimetric comparison with the leaves of the scale is n. As the absorption of the dye by single dead cells is not changed by light (as proved in the author's paper cited), the amount of the dye absorbed by dead cells is $\frac{21.6\,x}{22}$, and that absorbed by living cells is $\frac{6.4(100 - x)m}{78}$. At the same time, the whole amount of the dye absorbed is equal to $\frac{21.6n}{22} + \frac{6.4(100 - n)}{78}$. Thus $0.98x + 0.082(100 - x)m = 0.98n + 0.082(100 - n)$, or $x = \frac{0.9n - 8.2(m - 1)}{0.98 - 0.08m}$. When the permeability is not changed, that is, $m = 1$, $x = n$. The changes of the permeability were determined by the method of the absorption of methylene blue (see paper cited). After the exposure to light the leaves were transferred to the laboratory room so that the permeability might decrease again; thus the corrections of the number of dead cells given below are too great, that is, the true number lies between the observed and the corrected number.

[4] The standard solution of iodine contained one gram iodine and 3 grams potassium iodid in 100 cc.

[5] **Lepeschkin, W. W.** 1930. Light and the permeability of protoplasm. Amer. Jour. Bot. **26**: 208–218.

Usually 30 to 40 leaves cut from the same branch of *Elodea* were put into spring water, and then transferred into test tubes (capacity five cc.) containing about four cc. of spring water and made of Pyrex glass or quartz (no corks). Each tube contained five leaves. Two of the tubes were wrapped in black sateen (three layers) and all tubes were immersed in a water bath of an approximately constant temperature and exposed to direct sunlight. After the exposure the leaves were transferred into flasks containing a twelve percent solution of alcohol in distilled water and left there for exactly twenty minutes. Then the leaves were washed out in water and put in a 0.005 percent solution of methylene blue, to which 0.5 percent sodium chlorid was added to prevent an adsorption of the dye on the cell walls. After exactly ten minutes the leaves were investigated and the relative number of the cells was determined for each leaf. In some experiments a 0.02 percent solution of iodine was used, instead of alcohol. The leaves remained in this solution for ten minutes.

The energy of direct sunlight was measured by means of the radiometer described by Davis,[6] and was expressed in watt-hours per 10 sq. m., per 100 Å at a wave length of 0.500 μ. The energy of ultra-violet rays of sunlight is expressed in the same units.

The Influence of Visible Rays

Experiments on Yeast Cells

Two series of experiments were made. In one series the suspension of yeast cells was exposed to direct sunlight without the addition of alcohol. In the other, alcohol was added before the exposure to sunlight in amounts sufficient to check the synthetic processes and to kill the cells slowly. The concentration of alcohol added to the suspensions in the first case (after the exposure) was 23 percent, while the concentration in the second case was 17 or 18 percent. Water bath temperature was 25 to 26° C. The results are shown in tables 1 and 2.

Table 1. *Effect of Alcohol on Yeast after Exposure to Light*

Exper. No.	Exper. Began	Sunlight Energy	Exposure Period (minutes)	Percent of Dead Cells		
				Before	After	Control
1	Noon, May 6	92	60	45	100	50
2	Noon, May 7	92	60	50	100	54
3	2:30 P.M., May 7	80	60	46	65	45

The experiments 1, 2, and 3 showed that sunlight decreases the resistance of the protoplasm of yeast cells to alcohol.

In the author's papers cited above it was pointed out that *Spirogyra* possesses sufficient synthetical ability to restore the stability of its protoplasm. In the case of yeast cells this ability is also very high. As is

[6] **Davis, G.** Instruments for radiometric investigations, etc. Gen. Electric Review. February, 1931. 98–103.

known, yeast cells placed in a proper nutritive medium begin to multiply at once, but even in spring water the formation of buds is observed. It is therefore not surprising that yeast cells, suspended in spring water, are able to restore to some extent the stability of their protoplasm decreased by light. If the exposure to sunlight lasts for two hours, the restoration may sometimes be complete, as it is seen from experiments 4 and 5 (table 2).

If alcohol is added before the exposure to sunlight, the above restoration is checked and a decrease of resistance is observed even after two hours, as is seen from experiments 6 and 7 (table 2).

TABLE 2. *Effect of Alcohol on Yeast Exposed to Light*

Alcohol Added	Exper. No.	Exper. Began	Sunlight Energy	Exposure Period (minutes)	Percent of Dead Cells		
					Before	After	Control
After exposure	4	10 A.M., May 11	80	15	45	48	45
	4	10 A.M., May 11	80	30	45	75	45
	4	10 A.M., May 11	80	60	45	60	45
	5	11:30 A.M., May 12	77	120	50	45	22
Before exposure	6	11:20 A.M., May 14	78	120	2	13	6
	7	10:45 A.M., May 18	89	150	2	12	5

Experiments on Elodea

The experiments on *Elodea* leaves were made in April and May, 1931 and gave in general similar results to those on yeast cells; they showed that the resistance of protoplasm to alcohol and iodine is lower after the exposure to direct sunlight than before. This may be seen from the following tables. Water bath temperature was 25° C. In most experiments the colorimetric method was used. Thus in all tables two numbers of dead cells are given, one found by the colorimetric comparison (Obs.) and one corrected according to permeability changes (Corr.).

TABLE 3. *Effect of Poisons on Elodea Cells Exposed to Light*

Exper. No.	Exper. Began	Sunlight Energy	Exposure Period (minutes)	Poisons	Percent of Dead Cells				Method
					Before	After		Control	
						Obs.	Corr.		
8	11:30 A.M.	87	40	12% Alcohol	11	40	—	11	Direct
8	11:30 A.M.	87	40	12% Alcohol	11	40	34	9	Colorimetric
9	10:30 A.M.	92	25	12% Alcohol	6	14	10	5	Colorimetric
10	11:15 A.M.	92	180	12% Alcohol	15	50	—	10	Direct
10	11:15 A.M.	92	180	12% Alcohol	12	60	56	9	Colorimetric
11	10:00 A.M.	90	90	0.02% Iodine	15	40	34	12	Colorimetric
12	9:15 A.M.	60 to 92	500	0.02% Iodine	11	53	52	10	Colorimetric
13	Noon	92	26	12% Alcohol	12	22	20	10	Colorimetric
14	10:00 A.M.	85	120	12% Alcohol	17	55	53	14	Colorimetric

From these experiments it may be seen that the injuring effect of the exposure to direct sunlight increases rapidly during the first hour, so that the number of dead cells increases about three times. Further exposure

increases it, however, very slowly and even after an exposure of eight hours the amount of dead cells is increased by only one-third. This slow effect of sunlight may be ascribed to the synthetic activity of the cells, which restore the substances destroyed by light, although this restoration is not as active as in the case of yeast cells, and the resistance of protoplasm remains low if the exposure does not stop. On the other hand, transferring the leaves to the laboratory room (diffuse sunlight) stops the destruction, and the synthetic activity restores the former resistance of protoplasm in a comparatively short time, as is seen from the following experiments.

Experiment 15. An *Elodea* plant was cultivated on the window sill of the north side of the building in a crystallizing jar covered by a glass plate five mm. thick. At 9:30 A.M., April 17, five leaves from one branch of the plant were cut off and the resistance of their protoplasm to 0.02 percent iodine was determined; fifteen percent of dead cells were found. At 10:00 A.M. the jar with the plant was exposed to direct sunlight and immersed in the water bath (25° C.). At 11:00 A.M. the plant was brought to the laboratory room. Five leaves were cut and after the action of iodine, 38 percent of cells were dead. (The corrected number was 35 percent). The plant remained on the window sill until 12:15 P.M., when five more leaves were cut. After the action of iodine, 11 percent of the cells were dead. Three quarters of an hour was therefore sufficient for the plant to restore the stability of its protoplasm.

In the author's previous papers it was shown that the stability of protoplasm of *Spyrogyra* changes under the influence of diffuse sunlight if it affects the plant for some hours; moreover, daily variations of the stability are observed. A similar process could be detected also in *Elodea*, as is seen from the following experiments.

Experiment 16. At 10:30 A.M., April 10, leaves from one branch of *Elodea* were put into two crystallizing jars filled with water. One of them was wrapped in black sateen, while the other remained uncovered. Both were standing on the window sill on the north side of the building, in diffuse sunlight. In five and a half hours, the leaves of the first portion had 20 percent dead cells, while those of the second portion had 30 percent (direct method) after the action of alcohol (12 percent).

Experiment 17. The number of dead cells after the action of alcohol (12 percent) in the leaves of the same branch of *Elodea* cultivated in diffuse sunlight was as follows (colorimetric method):

Date	April 11		April 12		April 13	
Time	11:20 A.M.	4:56 P.M.	9:10 A.M.	5:43 P.M.	9:10 A.M.	4:30 P.M.
Percent of Dead Cells						
Observed	13	18	8	16	8	18
Corrected		16	10	13	10	16

Red and Blue-green-violet Rays

The results described above are due to the action of complete sunlight. Some experiments will be now described which determine whether short or long wave-lengths are more active in producing the decrease of the stability of protoplasm. The tubes containing the plant were put into metal boxes, open from one side, which were covered by a filter, and the boxes were immersed in the water bath (temperature 26° C.) and exposed to direct sunlight so that the tubes were illuminated through the filter. The filters, manufactured by Corning Glass Company, were the following: heat resisting red, G. 24, 2 mm. thick; and light-blue-green, G. 584, 4 mm. thick. The first transmits about 14 percent sun energy of wave-length 6000 Å to 7500 Å (max. 6800 Å), the second transmits about 25 percent energy of wave-length 3800 Å to 6000 Å (max. 4500 Å).

In the experiments on yeast cells, alcohol was added before the exposure to sunlight. The results are shown in table 4.

Table 4. *Yeast Cells Exposed to Red and Green-blue-violet Rays*

Exper. No.	Exper. Began	Sun Energy	Exposure Period, (minutes)	Alcohol %	Filter	Percent of Dead Cells		
						Before	After	Control
17*a*	11:20 A.M., May 15	92	120	17	Red	2	8.8	7
					Green-violet	2	17	7
18	10 A.M., May 19	80	120	18	Red	2	20	16
					Green-violet	2	29	16

If we divide the difference between the numbers of dead cells in the control tubes and in the illuminated tubes by the number of dead cells in the control tubes, we will obtain the relative increase of the number of dead cells produced by the illumination. If we now divide this relative increase of the number of dead cells by the amount of the energy transmitted by the filters expressed in percentage of the whole sun energy, we will obtain the activity of the rays. This activity for red rays is $\frac{1.8}{7 \times 14}$ (Exp. 17*a*) and $\frac{4}{16 \times 14}$ (Exp. 18); that is, at an average it is equal to 0.017. The activity for green-blue-violet rays is $\frac{10}{7 \times 25}$ (Exp. 17) and $\frac{13}{16 \times 25}$ (Exp. 18), or at an average 0.04. Therefore red rays are about half as active as green-blue-violet rays.

The experiments on *Elodea* leaves gave the following results shown in table 5. In these experiments the colorimetric method was used.

Table 5. *Elodea Leaves Exposed to Red and Green-blue-violet Rays*

Exper. No.	Exper. Began	Sun Energy	Exposure Period (minutes)	Alcohol %	Filter	Percent of Dead Cells			
						Before	After		Control
							Obs.	Corr.	
19	10 A.M., May 1	85	120	12	Red	20	22	22	16
					Green-violet	20	54	50	16
20	10 A.M., May 4	90	120	12	Red	17	18	16	

The activity of red rays for *Elodea* leaves is: $\frac{6}{16 \times 14}$ and $\frac{4}{14 \times 14}$ or an average of 0.023; and that of green-blue-violet rays is $\frac{34}{16 \times 25}$ and $\frac{52}{14 \times 25}$, or an average of 0.11. Thus in the case of *Elodea* the activity of green-blue-violet rays is about four times greater than that of red rays; that is, the difference between the action of the rays with short and long wave-lengths is still greater than in the case of yeast cells. This fact may be ascribed to the absorption of red rays by chlorophyll.

Ultraviolet Rays of the Sun Spectrum

In the author's previous paper [7] concerning the action of sunlight on red blood cells it was stated that, in contrast to visible rays, ultra-violet rays increase the stability of protoplasm if they act for a short time or are weak. They decrease the stability, however, if they act for a long time or are strong. Red corpuscles exposed to sunlight in quartz tubes showed no decrease and even an increase in their stability. The results of the experiments on *Elodea* exposed to sunlight in quartz tubes are seen from table 6. In these experiments the colorimetric method was used.

Table 6. *Elodea Leaves Exposed to Sunlight in Glass and Quartz Tubes*

Exper. No.	Exper. Began	Sun-light Energy	Exposure Period (minutes)	Poison	Tubes	Percent of Dead Cells			
						Before	After		Control
							Obs.	Corr.	
21	10:30 A.M., April 10	92	180	11% Alcohol	Glass	12	60	55	12
					Quartz		30	23	
22	Noon, April 20	92	30	11% Alcohol	Glass	12	22	14	12
					Quartz		16	7	
23	10:45 A.M., May 2	85	120	12% Alcohol	Glass	20	95	93	15
					Quartz		85	82	
24	9:00 A.M., April 18	87	90	0.2% Iodine	Glass	15	28	22	11
					Quartz		12	6	
25	10:00 A.M., May 4	85	120	12% Alcohol	Glass	19	55	50	14
					Quartz		68	62	

It may be seen from table 6 that in spite of a greater radiant energy received by the leaf cells in quartz tubes, the decrease of the resistance of their protoplasm to alcohol and iodine was in most cases smaller than in the glass tubes. Only in Experiment 25 was this decrease stronger. Therefore, in general, ultraviolet rays protected the leaf cells from the harmful effect of visible rays.

The protective effect of ultraviolet rays was observed also in the case of an exposure to diffuse sunlight. Thus, for instance, after the exposure to diffuse sunlight for 320 minutes, alcohol (12 percent) killed thirty percent of the leaf cells in glass tubes and twenty percent of the cells in quartz tubes (direct method).

[7] **Lepeschkin, W. W.** 1931. Haemolysis and changes in resistance of erythrocytes produced by light. Protoplasma 14: 11–27.

Similar results were obtained in the experiments on yeast cells, as may be seen from table 7.

TABLE 7. *Yeast Cells Exposed to Sunlight in Glass and Quartz Tubes*

Exper. No.	Exper. Began	Sun-light Energy	Exposure Period (minutes)	Alcohol Added	Tubes	Percent of Dead Cells		
						Before	After	Control
26	12:30 P.M., May 5	90	60	After, 23%	Glass	45	100	55
					Quartz		65	
27	2 P.M., May 11	83	30	After, 23%	Glass	46	71	50
					Quartz		50	
28	11:20 A.M., May 15	85	120	Before, 17%	Glass	2	13	7
					Quartz		8	
29	11 A.M., May 18	89	150	Before, 17%	Glass	2	12	6
					Quartz		10	
30	10 A.M., May 19	87	120	Before, 18%	Glass	20	40	20
					Quartz		50	

Experiments in which *Elodea* leaves were exposed to ultraviolet rays separated from the visible rays of sunlight showed that first an increase of the stability of protoplasm took place, then the increase was gradually transformed to a decrease which grew in time. The maximum of stability was observed after different periods of exposure, according to the strength of the ultraviolet rays. The separation of ultraviolet rays from visible light was made with the filter Purple-ultra G. 586A (Corning Glass Company) which transmitted less than two percent of solar energy of the wave-length 3200 to 4200 Å (max. 3700 Å) and about 10 percent of the whole energy of the ultraviolet rays. The results are given in table 8. The colorimetric method was used. The changes in permeability were indistinct (correction unnecessary).

TABLY 8. *Elodea Leaves Exposed to Ultraviolet Rays in Quartz Tubes (Filter G. 586A)*

Experiment 31 Noon, May 13	Exposure Period in Minutes...	0	5	10	20	40	105	200
Ultraviolet Energy 14	Percent of Cells Killed by 12% Alcohol.....	16	12	15	22	26	25	30
Experiment 32 10:30 A.M., May 14	Exposure Period in Minutes...	0	3	6	14	28	63	120
Ultraviolet Energy 12	Percent of Cells Killed by 12% Alcohol.....	15	12	12	8	14	15	20

In the experiment in which the whole amount of ultraviolet rays of sunlight was acting on protoplasm the period of exposure was much shorter. The preliminary experiments showed that the visible rays of sunlight produce no effect on the stability of protoplasm if the exposure is shorter than five minutes. It was therefore possible to investigate the action of ultraviolet rays without separating them from visible rays and exposing the quartz tubes with *Elodea* leaves or yeast to direct sunlight without any filters or a

water bath. The results are given in tables 9 and 10. In the case of *Elodea* the colorimetric method was used (no correction necessary).

TABLE 9. *Elodea Leaves Exposed to Direct Sunlight in Quartz Tubes (no Filter).*

Experiment 33 Noon, April 23	Exposure Period in Seconds..	0	5	15			
Ultraviolet Energy 14	Percent of Cells Killed by 12% Alcohol.....	38	16	33			
Experiment 34 11:00 A.M., April 25	Exposure Period in Seconds..	0	1	2	3	5	10
Ultraviolet Energy 8	Percent of Cells Killed by 11% Alcohol.....	12	12	7	8	3	7

TABLE 10. *Yeast Cells Exposed to Direct Sunlight in Quartz Tubes (no Filter)*

Experiment 35 10:30 A.M., May 9	Exposure Period in Seconds..	0	1	2	4	8	16	32	64
Ultraviolet Energy 12	Percent of Cells Killed by 23% Alcohol after Exposure.....	45	46	32	32	32	28	30	54

The maximum of the stability of protoplasm of *Elodea* was reached, therefore, in four to five seconds. The energy of ultraviolet rays received by the leaves was about ten times greater than the energy received by the leaves through the filter, in which case the maximum of stability was reached in five to fourteen minutes. This result might show that the ultraviolet rays which were absorbed by the filter (wave-length below 3200 Å) are probably the most active in producing an increasing effect on the stability of protoplasm. The action of these rays may be seen from the following experiments.

Elodea leaves were exposed in quartz tubes to the ultraviolet rays of wave-length 3100, 3030, 2970, and 2920 Å. For this purpose the sun spectrum of the spectrograph of the Desert Sanatorium was used. This instrument was recently described by Davis (l. c.). The results are given in table 11.

TABLE 11. *Elodea Leaves Exposed to Ultraviolet Rays of Sun Spectrum (no Water Bath)*

Exper. No.	Exper. Began	Exposure Period in Minutes	Percent of Cells Killed by Alcohol (12%) after Exposure to Wave-lengths of:				Control	Ultra-violet Energy
			3100 Å	3030 Å	2970 Å	2920 Å		
36	Noon, April 18.....	5	60	45	28	85	30	14
37	Noon, April 23.....	1.5	15	17	22	40	38	14
38	Noon, May 2.....	0.5	20	20	12	14	28	14

From these experiments may be seen that the exposure to the rays of a wave-length 3100, 3030, and 2920 Å for five minutes decreased the stability

of protoplasm, while the ray 2970 Å increased it. If the exposure was shortened to 1.5 minutes all rays except 2920 Å increased the stability. At last even these rays produced an increase of stability if the exposure was 0.5 minutes. At all events, the rays of a wave-length 2970 Å are the most active in producing an increase of stability, while the most destructive are the rays of wave-length 2920 Å.

It is very probable that the protective and injurious effects of ultraviolet rays are independent of each other. These rays may even protect protoplasm from their own destructive action. At all events, the comparison of the results given in tables 8, 9, 10, and 11 with those given in tables 6 and 7 shows that the protective effect is still distinct when the exposure lasts for such a long time that ultraviolet rays become harmful. Although the discrepancy between the results given in tables 6 and 8 may also be explained by the absence (among the rays transmitted by the filter used) of the rays most effective in producing an increase of the stability of protoplasm, the comparison of the results given in tables 6 and 9 or 7 and 10 shows that after the maximum of stability had been reached this stability decreased but remained in most cases greater than the stability in glass tubes (transmitting visible light). Ultraviolet rays penetrating protoplasm evidently produce some very rapid changes there, which remain during the whole exposure and which to some extent protect protoplasm from the injurious effect of visible rays. In the case of red blood cells this protective effect is so great that during the first two hours of exposure the stability of protoplasm usually remains greater than normal.

It is quite impossible at present to give an entirely satisfactory explanation of the protective action of ultraviolet rays on protoplasm. Considering the fundamental work done by Clark,[8] however, we would suggest that ultraviolet rays probably throw out electrons from the protein part of protoplasm and make the latter more alkaline. A slightly alkaline reaction is known as favorable to living processes, and in the author's papers cited above it was shown that alkaline reaction increases the resistance of *Spirogyra* protoplasm to mechanical injuries and high temperature. It may also protect protoplasm from the harmful effect of visible light and poisons.

Summary

1. Visible rays of direct sunlight decrease the stability of living matter. This decrease is also produced by diffuse sunlight but the exposure must be longer. At night the stability of protoplasm is greater than by day.

2. The resistance of living matter to alcohol and iodine, after exposure to light, was investigated on yeast cells and leaf cells of *Elodea*.

3. The decrease of the stability of living matter produced by direct sunlight disappears soon in diffuse light because synthetic processes of the

[8] **Clark, Janet H.** 1922. Amer. Jour. Physiol. **61**: 72–79. 1925. Ibid. **73**: 649–660.

cell restore substances changed by light. These processes in yeast cells are so effective that the decrease of the stability completely disappears in two hours in spite of the continued illumination by direct sunlight. If the synthetic processes are depressed by alcohol, the stability of protoplasm decreases further.

4. Among visible rays of sunlight, green-blue-violet rays are more effective in producing the decrease of the stability than red rays.

5. Ultraviolet rays produce an increase of the stability of living matter. The maximum stability is reached in some seconds or minutes according to their strength, but their protective effect on protoplasm is still distinct after two and more hours of exposure to sunlight.

6. If ultraviolet rays act for a long time or are strong, they decrease the stability of protoplasm as visible rays do. The protective and harmful effects of ultraviolet rays are independent of each other.

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CITRIC ACID PRODUCTION BY *ASPERGILLUS NIGER*[1]

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A detailed review of the literature concerning the production of acids by fungi has been presented by Bernhauer and Schoen (2), Challenger (10), Schober (18), and Chezaszcz and Tiukow (11). A number of theories concerning the mechanism of citric acid formation have been proposed. These may be summarized as follows:

1. The acid is a product of proteolysis and is a residue in the decomposition of amino acids (14, 15).

2. The acid is a result of a synthetic process, after the sugar is broken down to shorter chains of carbon. Thus the units may be acetaldehyde (13), oxalic and acetic acids (4), or acetic acid (11).

3. The acid is formed by an inner aldolization process, whereby a five-carbon ring is produced which oxidizes to citric acid (6) (7).

4. The acid is a result of progressive oxidation of sugar to gluconic, saccharic, adipic, and then to citric acid (8, 9, 10).

METHODS

One-hundred cc. portions of the experimental solution were placed in 250 cc. Erlenmyer flasks and sterilized for 20 minutes at 10 pounds pressure. After cooling, these were inoculated with a spore suspension and incubated at 28°–30° C. for the allotted periods of time. Upon removal from the incubator, the weight of fungus, sugar, total acidity, citric and oxalic acids were determined. Each test was run in duplicate, and the data given in the tables are the average values of the two flasks. The values of these similarly treated flasks approximated each other.

Total acidity was obtained by titrating an aliquot with 0.1*N* NaOH, using phenolpthalein as the indicator.

The oxalic and citric acids were determined gravimetrically as the calcium salts according to the method of Bernhauer (1). The culture solution was made up to 250 cc. and 50 cc. removed for analysis. To this aliquot 10 cc. of 10 percent $CaCl_2$ were added, and neutralized with 10 percent NH_4OH. Two cc. of a 20 percent HCl solution were then added and the solution heated for a short while. This was then allowed to cool and the calcium oxalate was filtered through a tared Gooch crucible and washed with cold water. The salt was dried at 100° C. and weighed as $Ca(OOC)_2.H_2O$, which contains 61.62 percent water-free oxalic acid.

The filtrate and the washings were concentrated and, while hot, ammonium hydroxid was added to bring about neutralization. Concentration

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

was continued to a volume of 25–30 cc. The solution was filtered hot through a tared Gooch crucible, washed with hot water, and then with 60 percent alcohol. The salt was dried at 110°–115° C. and the calcium citrate thus obtained was multiplied by 0.7709 to give the amount of citric acid. By this method 96 percent of the theoretical value of the acids was recovered.

The organism used for these studies is designated as *Aspergillus niger No. 5* from the collection kept by Dr. S. A. Waksman at the New Jersey Agricultural Experiment Station. This strain had previously given indications of a high acid production and an opportunity was taken to find the optimum conditions for a high citric acid yield, such yield to be obtained in a shorter period of time.

Experimental Results

The ability of the fungus to produce acid was first ascertained by growing it in the following medium: cane sugar, 200 gm.; NH_4NO_3, 1.0 gm.; K_2HPO_4, 0.1 gm.; KCl, 0.1 gm.; $MgSO_4.7H_2O$, 0.1 gm.; $FeCl_3$, 0.02 gm. and water to make one liter. The amount of titratable acid was highest after seven days, reaching a value equivalent to 30 cc. 0.1*N* per 100 cc. of culture solution. This preliminary work was followed by a study of the influence of the various salts upon the production of citric acid.

Influence of Nitrogen Salts upon the Production of Citric Acid

Inorganic nitrogen salts were used in different amounts, as indicated in table 1, with the other ingredients remaining the same as given above. The solutions were removed after 4, 7, and 12 days, but as the short incubation period gave only small amounts of citric acid these values are not recorded in the table.

Table 1. *Influence of Inorganic Salts Containing Nitrogen upon Citric Acid Production by Aspergillus niger on a 20 Percent Sugar Solution*

Nitrogen Source	Amount (gm.)	Incubation Period (days)	Fungus Growth (gm.)	Titratable Acidity *N*/10 (cc.)	Citric Acid Found (gm.)	Sugar Disappeared from Solution (gm.)	Utilized Sugar Converted to Citric Acid (%)	Total Sugar Converted to Citric Acid (%)
$(NH_4)_2SO_4$	0.25	7	0.24	44	0.027	4.2	0.64	0.14
		12	0.25	39	0.067	4.9	1.36	0.34
	1.00	7	0.64	104	0.294	6.5	4.52	1.47
		12	0.69	111	0.359	8.8	4.08	1.79
	3.00	7	2.12	192	0.684	8.6	7.95	3.42
		12	2.04	205	0.864	10.8	8.00	4.32
NH_4NO_3	0.15	7	0.40	52	0.078	3.9	2.00	0.39
		12	0.36	50	0.039	7.8	0.48	0.20
	0.50	7	0.61	104	0.374	4.8	7.79	1.87
		12	0.58	90	0.327	10.2	3.20	1.64
	4.00	7	2.03	168	0.891	5.4	16.50	4.46
		12	2.00	122	0.579	11.2	5.17	2.90
$NaNO_3$	0.30	7	0.21	69	0.139	5.4	2.57	0.70
		12	0.44	58	0.019	5.4	0.35	0.09
	1.20	7	0.60	158	0.590	5.7	10.35	2.95
		12	0.77	158	0.632	9.3	6.79	3.16
	4.00	7	2.33	304	1.590	9.8	16.23	7.95
		12	1.90	289	1.455	12.4	11.73	7.28

It is noticeable that the highest yield of citric acid was obtained with 4.0 gm. $NaNO_3$ per liter and not with NH_4NO_3 as has been reported previously (12). An incubation period of 7 days gave the best yield with about 16 percent of the sugar that had disappeared being utilized. Increasing the incubation period tended to decrease the amount recovered.

In a similar manner it was determined that for the maximum yield of citric acid the optimum amounts of the salts per liter were: K_2HPO_4, 1.0 gm.; KCl, 0.5 gm.; $MgSO_4.7H_2O$; 0.5 gm.

Influence of $FeCl_3$

As shown in table 2, the presence of $FeCl_3$ affected the formation of acid. The acidity and weight of the fungus reached a maximum when 0.02 gm. FCl_3 was added per liter. These values then decreased with higher concentration of iron and a distinct toxic effect was noticeable with the greatest amount of $FeCl_3$ used. Thus, the addition of iron increased the growth until the optimum was reached, but at the same time waste products per unit of growth showed only slight variations as indicated by the acidity of the solution.

TABLE 2. *Influence of $FeCl_3$ upon the Production of Acid by Aspergillus niger in a 20 Percent Sugar Solution in Seven Days*

$FeCl_3$ per Liter (gm.)	Fungus Growth (gm.)	Titratable Acidity $N/10$ (cc.)
0.00	0.67	91
0.01	1.74	270
0.02	2.73	370
0.05	2.60	364
0.10	2.53	323
0.20	0.36	48

Influence of Other Metallic Salts

In order to test the action of certain other metallic elements upon the yield of citric acid by the fungus under consideration, concentrations of 0.01 gm. $ZnSO_4$, $MnSO_4$, $NiSO_4$, and $CuSO_4$, per liter of medium were tried, and the results obtained are presented in table 3. The zinc salt gave the most marked effect, increasing the actual yield of citric acid as well as the amount of acid obtained per unit of sugar used. The other elements proved inhibitory at these concentrations.

TABLE 3. *Influence of Various Metallic Salts, 0.01 gm. per Liter, on the Production of Citric Acid by Aspergillus niger in a 20 Percent Sugar Solution, in Seven Days*

Salt	Sugar Consumed (gm.)	Fungus Growth (gm.)	Acid Production			Utilized Sugar Changed to Citric Acid (%)	Total Sugar Recovered as Citric Acid (%)
			Oxalic Acid (gm.)	Citric Acid (gm.)	Titratable Acidity $N/10$ (cc.)		
None	9.8	2.32	0.218	1.600	300	16.32	8.00
$NiSO_4$	5.0	0.40	0.029	0.213	72	4.26	1.01
$MnSO_4$	5.0	0.72	0.092	0.367	102	7.34	1.84
$CuSO_4$	7.0	0.97	0.132	0.093	119	1.33	.47
$ZnSO_4$	9.0	2.94	0.341	1.850	400	20.55	9.25

The amounts of acid recovered as citric and oxalic in the solution which did not contain the heavy metals show good agreement with the titratable acidity. In the other solutions, the acids identified did not account for the total acidity. This is presented more clearly in table 4. As interest lay in obtaining high yields of citric acid, no quantitative tests were made for other organic acids that are produced by fungi. Gluconic acid was identified, and this apparent discrepancy between the titratable acidity and recovered acids must be due to the various acids produced in the medium as the result of side reactions.

TABLE 4. *Differences in Titratable Acidity and Identified Acids in a 20 Percent Sugar Solution*

Salt Used	Titratable Acidity N/10 (cc.)	Oxalic Acid		Citric Acid		Acidity Accounted for (cc.)	Acidity Unaccounted for (cc.)
		Found (gm.)	Equivalent N/10 (cc.)	Found (gm.)	Equivalent N/10 (cc.)		
None	300	0.218	48.5	1.600	250.0	298.5	1.5
$NiSO_4$	72	0.029	6.4	0.213	33.3	39.7	32.3
$MnSO_4$	102	0.092	20.5	0.367	58.8	79.3	22.7
$CuSO_4$	119	0.132	30.0	0.093	14.5	44.5	74.5
$ZnSO_4$	400	0.341	75.8	1.850	289.1	364.9	35.1

Influence of Zinc Concentration

The addition of $ZnSO_4$ to a solution has been shown to increase fungus growth and, at certain concentrations, to inhibit spore formation (17); in fact it has been stated that zinc is essential for the proper metabolism of the fungus (3). Steinberg (19), working with two strains of *Aspergillus niger*, found that the minimum amount of zinc necessary to obtain maximum growth varied with the strain, and that the addition of both iron and zinc gave the greatest growth. In media prepared for citric acid production, Molliard (16) added 0.026 gm. $ZnSO_4$ per liter, while Bernhauer (1) used 0.05 gm. and Butkewitsch (5) 0.1 gm., whereas Currie (12) believed zinc unessential. Such differences in opinion as to the efficacy of this element made the experiment to determine the optimum concentration of $ZnSO_4$ necessary. Concentrations of this salt varying from 0.01 to 0.10 gm. per liter were prepared, inoculated, incubated, and analyzed with the results recorded in table 5. Increasing the amount of zinc increased the yield of

TABLE 5. *Influence of Zinc Sulfate upon the Production of Citric Acid by Aspergillus niger in a 20 Percent Sugar Solution*

Amount of $ZnSO_4$ per Liter (gm.)	Incubation (days)	Sugar Consumed (gm.)	Fungus Growth (gm.)	Acid Production			Utilized Sugar Converted to Citric Acid (%)	Total Sugar Recovered as Citric Acid (%)
				Oxalic Acid (gm.)	Citric Acid Found (gm.)	Titratable Acidity N/10 (cc.)		
0.01	4	7.93	1.33	0.201	1.314	267	16.57	6.06
	7	10.12	2.18	0.293	2.338	452	23.10	11.69
0.05	4	7.03	1.48	0.214	1.257	256	17.88	6.29
	7	10.62	2.13	0.315	2.317	460	21.80	11.59
0.075	4	7.73	1.56	0.235	1.440	287	18.63	7.20
	7	9.77	2.08	0.320	2.292	455	23.35	11.46
0.10	4	8.48	1.56	0.237	1.489	296	17.56	7.45
	7	10.75	2.17	0.319	2.540	495	23.63	12.70

citric acid from 8 percent of the available sugar where no zinc was added (table 3) to 12.7 percent where 0.10 gm. zinc was added per liter. A solution containing 0.01 percent $ZnSO_4$ gave almost as good results in 4 days as the zinc-free solution gave in 7 days.

The growth in the presence of increments of zinc showed marked differences. The dark spores were replaced by spores of a gray to green tinge and in the higher concentration spore formation was almost totally repressed. At the same time the mat showed heavy corrugations which folded deeply into the liquid. Evidently, the presence of zinc in the solution favored the vegetative processes in the fungus and accelerated the production of citric acid.

Another series of flasks was prepared containing $ZnSO_4$ in concentrations up to 0.5 gm. per liter. The acidity was practically alike in those concentrations up to 0.3 gm. per liter and then began to drop off. As 0.1 gm. $ZnSO_4$ gave the best yield this amount was used in the following experiment.

The Influence of Sugar Concentrations

The sugar content of the solutions commonly used by investigators varied from 5 to 20 percent. In order to determine the concentration of sugar that will give the greatest citric acid yield with the particular strain of *Aspergillus niger*, different amounts of sugar were used as indicated in table 6.

TABLE 6. *Influence of Sugar Concentration upon the Production of Citric Acid by Aspergillus niger in Seven Days*

Sugar Concentration (%)	Sugar Consumed (gm.)	Fungus Growth (gm.)	Acid Production			Utilized Sugar Converted to Citric Acid (%)	Total Sugar Recovered as Citric Acid (%)
			Oxalic Acid (gm.)	Citric Acid Found (gm.)	Titratable Acidity N/10 (cc.)		
5	4.83	1.58	0.422	0.046	114	0.95	0.92
10	7.81	2.55	0.463	1.216	248	15.57	12.13
15	9.85	2.67	0.259	2.375	416	24.11	15.83
20	10.15	2.85	0.159	2.726	503	26.66	13.63

The amount of sugar consumed increased with an increase in the amounts available to the organism. However, there was no direct ratio, for of the 20 gm. available, 10.15 gm. were used. One would then be led to believe that if 10 gm. of sugar were available all would be utilized, but this was not the case, since only 7.81 gms. had disappeared. Using a 20 percent sugar concentration gave the highest titratable acidity per unit of sugar utilized and in this solution the highest amount of utilized sugar was converted to citric acid, but the recovery based on the total sugar present was less than in the solution containing 15 percent sugar. The 5 percent sugar solution gave an extremely low recovery of citric acid, and is the result of insufficient sugar and the subsequent utilization of the citric acid by the fungus. The necessity of high concentrations of sugar is clearly indicated.

The foregoing experiments have shown that *Aspergillus niger* gave a high yield of citric acid when grown in the following culture solution for 7 days at 28°–30°:

Sugar (sucrose)	150–200.00 gm.
Sodium nitrate ($NaNO_3$)	4.00 gm.
Di-potassium phosphate (K_2HPO_4)	1.00 gm.
Magnesium sulfate ($MgSO_4.7H_2O$)	0.50 gm.
Potassium chlorid (KCl)	0.50 gm.
Ferric chlorid ($FeCl_3$)	0.02 gm.
Zinc sulfate ($ZnSo_4$)	0.10 gm.
Water to make up to	1 liter

Acid Formation as Influenced by Aeration

As citric acid in an oxidation product of a hexose ($C_6H_{12}O_6 + 1\frac{1}{2}O_2 = C_6H_8O_7 + 2H_2O$) it was thought that aeration would increase the acid yield. Flasks were so arranged that when suction was applied a continuous flow of cotton-filtered air was passed through the solution. With one series, air was bubbled through immediately upon inoculation; while with another series, the growth was allowed to form over the surface before the solution was aerated. The liquid was covered with a thin mat in 48 hours.

TABLE 7. *Influence of Aeration upon Acid Formation by Aspergillus niger*

	Incubation (days)	Sugar Consumed (gm.)	Fungus Growth (gm.)	Titratable Acidity N/10 (cc.)
Aeration	4	8.4	0.18	22
Continuous	7	8.6	0.40	28
Aeration after pellicle formation	7	10.0	0.49	26.5

As can be seen from table 7, the amount of sugar that had disappeared was large, but the acid yield was negligible when compared to that previously obtained. The organism showed but slight growth; evidently oxidation had proceeded too far, the sugar having been broken down completely, instead of to the intermediate acids stage.

Other Experiments on the Production of Acid by Aspergillus niger

Currie (12) suggested the possibility of renewing the solution and allowing the same fungus growth to continue its action, while Bernhauer (1) conducted his experiments on gluconic acid production in a similar manner. In the following experiment, a similar plan was tried in which the fungus was permitted to continue its activity on sugar solutions in the presence and absence of nutrient salts. This method yielded only small amounts of citric acid in the renewed solutions with most of the sugar remaining unused. The production of this acid must be linked with the early processes of growth.

A study of acid formation by reinoculating the spent solution after a greater part of the acid was neutralized with $CaCO_3$ was then tried. The yield of citric acid was greatest in the first period of 7 days, while in the reinoculated solutions this acid showed practically no increase.

Comparison of Various Media for Citric Acid Production

When comparing two other media that have been extensively used for the production of citric acid by fungi with the one proposed in this article, marked differences were noted. The solutions proposed by Currie (12) and Bernhauer (1) were studied for the comparative purposes.

Currie made use of the following solution: 150 gm. sucrose; 2.5 gm. NH_4NO_3; 1.0 gm. KH_2PO_4; 0.25 gm. $MgSO_4.7H_2O$; 5 cc. HCl (to pH3.5); water to 1000 cc.

Bernhauer used the following solution: 100 gm. sucrose; 2 gm. NH_4NO_3; 0.5 gm. KH_2PO_4; 0.5 gm. $MgSO_4,7H_2O$; 0.05 gm. $FeCl_3$; 0.05 gm. $ZnSO_4$.

TABLE 8. *Citric Acid Production in Various Solutions by Aspergillus niger*

	Sugar Content (%)	Sugar Consumed (gm.)	Incubation Period (days)	Fungus Growth (gm.)	Titratable Acidity N/10 (cc.)	Citric Acid (gm.)	Oxalic Acid (gm.)	Utilized Sugar Converted to Citric Acid (%)	Total Sugar Recovered as Citric Acid (%)
Currie's solution	15	4.69	7	1.89	160.0	0.840	0.004	17.9	5.6
		8.18	10	2.75	374.6	2.310	0.035	28.2	15.4
Bernhauer's solution	10	5.00	7	1.81	141.0	0.775	0.040	15.5	7.8
		5.32	10	1.89	173.9	0.989	0.054	18.6	9.9
New solution	20	8.99	7	2.52	503.0	2.726	0.317	30.3	13.6
		8.94	10	2.66	505.7	2.880	0.356	32.2	14.4

The results obtained as shown in table 8 gave the effect of $ZnSO_4$ on the production of citric acid. Although Currie's solution gave a high yield of the acid in 10 days, the new solution gave almost the same yield in 7 days when figured on the percent of sugar recovered. In fact, in some of the flasks the percent recovery was comparable to those obtained by Currie. The actual yield of citric acid is much greater in the new solution. Bernhauer's solution is not as efficient as either of the other two. The yield in the zinc-containing solution was not increased markedly by permitting the flasks to remain in the incubator for 10 days. Thus, the process of citric acid production by fungi can be shortened by 3 days.

DISCUSSION

The *Aspergillus niger* used throughout these investigations produces the greatest amount of citric acid when $NaNO_3$ and not NH_4NO_3 is used as a source of nitrogen. In fact, all media that have been proposed for high yields of this acid contain the nitrate radical, and undoubtedly nitrogen in such a form is prerequisite for the production of large amounts of citric acid. Although oxygen is necessary, yet the supply must be limited; otherwise, with an unlimited supply of oxygen the citric acid does not accumulate but is broken down to CO_2 and water as is indicated by the aeration experiment. The acid is not found in large amounts until a heavy mat of mycelium has

been produced over the surface of the medium. Such a heavy growth tends to limit the supply of available oxygen within the solution and favors the partial oxidation of the sugar to citric and other organic acids.

The production of citric acid is evidently dependent on the early growth processes of the fungus. If a heavy fungus mat forms rapidly on the surface of the solution, high acidity may be expected. The proper concentration of iron favors such rapid and heavy growth. Of the various other heavier metals, zinc gives increased yields of citric acid. The type of growth is greatly modified by the addition of zinc, showing very few dark spores and a heavy vegetative growth with wrinkles and folds. Such a type of mycelial development, which is essential to high acid yields, is also favored by high concentrations of sugar. The depression of spore formation and the promotion of mycelial or vegetative growth is a necessity for high citric acid recovery.

Summary

1. A strain of *Aspergillus niger* which had been isolated from the soil was studied as to its ability to produce citric acid in a sugar solution, and an increase in 0.1*N* titratable acidity from 30 cc. to 500 cc. per 100 cc. culture solution was obtained by the modification of the original solution.

2. Sugar concentrations of 15 to 20 percent were necessary for high yields of citric acid, although the sugar was not completely used.

3. A 5 percent concentration of sugar gave small yields of citric acid, as this acid was used as an energy source by the organism.

4. As a source of nitrogen, $NaNO_3$ was far superior to NH_4NO_3 or $(NH_4)_2SO_4$ for citric acid production.

5. The presence of iron and zinc was essential for rapid growth and increased citric acid accumulation.

6. Zinc especially favored such acid production which was accompanied by the absence of dark spores and the increased development of mycelial or vegetative growth.

7. Renewing the spent solution under the hyphal mat gave no increased acid yields.

8. Aerating the solution permitted no accumulation of the citric acid.

9. The greatest yield of citric acid was obtained after an incubation period of 7 days.

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THE INFLUENCE OF NARCOTICS, MECHANICAL AGENTS, AND LIGHT UPON THE PERMEABILITY OF PROTOPLASM

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Some facts observed by the author (9, 12) caused him to conclude that not only the surface of protoplasm but its whole mass is responsible for the peculiarities of osmotic processes in the cell, although different parts of protoplasm may have different osmotic properties. We may expect, therefore, that every chemical change brought about in protoplasm, every dissolving of foreign substances in it or a removal of substances from it, may influence its osmotic properties.

Thus, it is not surprising that light, which produces a photochemical change in protoplasm (13), brings about an increase of its permeability. It is also comprehensible that narcotics change its permeability. They can produce this change, for instance, if they dissolve in protoplasm or if they remove lipoids from it. In both cases the solvency of protoplasm is changed. In the first case a decrease of the permeability to substances insoluble in narcotics may be expected; in the second case an increase of the permeability to substances insoluble in lipoids may be observed. The author's experiments showed, indeed, that low concentrations of narcotics decrease the permeability, while high concentrations increase it (5, 6). This result was confirmed later by several investigators. The literature may be found in Winterstein's monograph of narcosis (18). An increase of permeability produced by ether was also recently observed by Weber and Höfler (17).

In the present paper experiments are described in which some details of the action of narcotics on the permeability of protoplasm are elucidated. In connection with their increasing effect on this permeability, which doubtless should be ascribed to their destructive effect on protoplasm (compare, for instance, evidences mentioned in Winterstein's monograph), there is a destructive effect of mechanical agents on protoplasm. Special experiments were therefore necessary to investigate these effects. It was also important to determine whether narcotics influence the permeability changes produced by light. As is known, narcotics check some processes of metabolism (for instance, photosynthesis and respiration); one might expect, therefore, that they may also check the changes of permeability. Indeed, Tröndle (14) described some experiments to prove that the increase of permeability in light does not occur in the presence of narcotics. According to my opinion, however, these experiments really show only that the permeability of

protoplasm is less in the presence of narcotics in light. To support this opinion some experiments on the simultaneous effect of narcotics and light are described in the present paper.

Material and Methods

Two methods for the determination of the permeability of protoplasm were used. One was that of the absorption of aniline dyes, the other that of isotonic coefficients. The experimental material was leaves of *Elodea canadensis* and the epidermal cells from the lower side of the main vein of the leaves of *Tradescantia* (*Rhoeo*) *discolor*. *Elodea* was used in both methods, while *Tradescantia* only in the method of isotonic coefficients. The colorimetric method of the absorption of aniline dyes in application to *Elodea* was recently described by the author (13). The method of isotonic coefficients, which was described by the author more than twenty years ago (4), may be considered the best among the so-called osmotic methods for the determination of permeability (7). The critics of the method did not use it in a proper manner and secured inexact results. Thus, some important details of this method should be emphasized here.

The osmotic pressure of an aqueous solution does not depend upon the properties of the membrane if the membrane is impermeable for the solute. If the membrane (in our case the protoplasmic layer separating the cell sap from the cell membrane and the outer solution) is permeable for the solute, the osmotic pressure (that is, the force necessary to separate the solute from the solvent by means of the membrane) depends upon the permeability of this membrane to the solute. If we plasmolyze the same cell with solutions of two substances, one penetrating comparatively easily through protoplasm and the second not penetrating, we shall find that the theoretic osmotic pressure (calculated from freezing point, for instance) of the solution of the first substance, producing the same degree of plasmolysis as the solution of the second substance, is greater than the theoretical osmotic pressure of the latter. The real osmotic pressure of the first solution P, that is the pressure equal to the pressure of the second solution, can be calculated from the formula $P = P_0(1 - \mu)$, where P_0 is the theoretic osmotic pressure and μ is the permeability factor which may be considered proportional to the permeability of protoplasm to the first substance. This factor can be calculated from the formula $\mu = 1 - \frac{C}{C'}$, where C is the concentration of the easily penetrating substance (found from physico-chemical tables) the solution of which has the same osmotic pressure as the solution of the substance which does not penetrate and produces plasmolysis, and C' is the experimentally determined concentration of the easily penetrating substance. The above formula is an approximate one, but it permits the detection of changes of permeability which are greater or smaller than 3 to 20 percent of its initial value (7).

In the present experiments sucrose and potassium nitrate were used as plasmolyzing substances. Both were obtained from Merck, dried in a desiccator, and dissolved in water distilled in a glass still, the pH of which as well as that of the solutions was always adjusted to 7.3 by an addition of NaH_2PO_4 and Na_2HPO_4 in an amount smaller than 0.001 gram-mol in 1000 cc. The standard solutions contained 0.5 gram-mol of sucrose or potassium nitrate in 1000 cc. in the case of *Elodea*, and 0.3 gram-mol in 1000 cc. in the case of *Tradescantia;* they were kept in bottles with ground glass stoppers in a refrigerator and diluted with distilled water before experiments. The concentrations of plasmolyzing solutions were first determined approximately, then two sets of solutions with concentrations between the found limits ("no plasmolysis" and "all cells plasmolyzed") were made. The difference between neighboring concentrations in the set were 0.005 gr. mol. for KNO_3 and 0.01 gr. mol. for sucrose in the case of *Elodea;* 0.002 gr. mol. for KNO_3 and 0.004 gr. mol. for sucrose in the experiments on *Tradescantia*. Each set usually contained six concentrations, but sometimes ten. The solutions were poured into specimen vials closed with corks.

In most experiments cut leaves of *Elodea* or slices of *Tradescantia* were kept in spring water (pH = 7.3) for about ten hours before using them. *Elodea* leaves were cut from the same branch of the plant, while the epidermal slices were taken from the same spot of *Tradescantia* leaf. In order to determine the permeability factor in the case of *Elodea* about 12 leaves were put into solutions of KNO_3 of different concentrations. As the water should be removed from the wet leaves by blotting paper, the operation took about three minutes. In seven minutes the leaves were investigated under the microscope beginning with the leaves kept in the greatest concentration. In 20 minutes this investigation was finished, and the leaves were transferred to the sugar solutions; their investigation under the microscope began in 30 minutes and was finished in 40 minutes. The indicated periods of time were sufficient for the settlement of the osmotic equilibrium.[1] A concentration in which half of the cells of a leaf were plasmolyzed was considered to be isotonic. If the number of plasmolyzed cells was smaller than that of non-plasmolyzed cells, the next greater concentration in the set was considered as isotonic. If the first number was greater than the second, the next smaller concentration was considered as isotonic. If almost all cells were plasmolyzed or not plasmolyzed, the leaf was transferred into a smaller or greater concentration, and investigated in 5 minutes (KNO_3) or in 10 minutes (sucrose) once more. Only the cells of the lower layer of the leaf were considered.

The concentration of the cell sap of *Tradescantia* epidermis is smaller and varies more than that of *Elodea* leaves. Thus the isotonic concentrations of KNO_3 and sucrose had to be determined on the same slice. Usually

[1] If the leaves remained longer in KNO_3 solutions, an increase of the volume of protoplast was observed (penetrating of KNO_3 into the cell sap).

four to five slices were put into solutions of KNO_3. In 15 minutes the microscopic investigation began, and it was finished in 30 minutes. Then the slices were transferred into sucrose solutions. The form of the slices was drawn on paper in order not to confuse them. The slices remained in the sucrose solutions for about 30 minutes, then the microscopic investigation began, and in 40 to 50 minutes the isotonic concentrations were determined. As isotonic was considered the concentration in which half of the number of cells of a slice were plasmolyzed. It is very important that in the case of *Tradescantia* the isotonic coefficients are determined on each slice separately. In the case of a greater concentration of cell sap and a smaller variation of this concentration in different cells (*Elodea*) average isotonic concentrations of KNO_3 and sucrose for several leaves may be determined, but even in this case the same leaves for the determination of the isotonic concentration of KNO_3 and sucrose should be used. Only this manner of determination of the permeability factors gives an exact result. It is therefore not surprising that Zycha (19) could not detect any permeability change using the method of isotonic coefficients in a wrong manner.

This method of determination of permeability is better than other osmotic methods because the cells are submitted to a harmful influence of salt solutions for a much shorter time (20 to 30 minutes), and the whole process of determination of permeability requires only one to one and a half hours, while in other osmotic methods the cells are often immersed in a salt solution for several hours.

Permeability and Narcotics

As has been mentioned narcotics decrease the permeability of protoplasm for salts and some other water-soluble substances if they act in comparatively small concentrations, while they increase this permeability if taken in greater concentrations.[2] This double action of narcotics, however, is explained differently by different investigators. We may consider first the decreasing effect on permeability.

The author's explanation was quite simple: ether and chloroform dissolve in liquid protoplasm and, as they do not dissolve salts and some other water soluble substances, they in general decrease the solubility of these substances in protoplasm; hence, the permeability decreases. Winterstein (18) thinks however that narcotics are adsorbed on the surface of colloidal particles of protoplasm, increasing their volume; the intervals between the particles decrease and penetrating substances are hindered on their way through protoplasm. This opinion was probably based on the fact that narcotics decrease the diffusion through artificial solid membranes. Protoplasm is liquid, however; thus every increase of the volume of its dispersed phases

[2] It is evident that some intermediate concentration of a narcotic does not influence the permeability at all. Thus it is not surprising that Ruhland could not find any influence of ether on the permeability of *Spirogyra* protoplasm (Jahrb. Wiss. Bot. 51: 376, 1912).

should lead to an increase of its viscosity. Heilbronn (2) and Weber (15, 16) found that the viscosity of protoplasm is decreased by small concentrations of narcotics. Therefore, Winterstein's opinion is scarcely correct.

In the author's former paper on the action of narcotics on the permeability of protoplasm it was pointed out that chloroform and ether do not diminish permeability to the aniline dye Bismark brown, which dissolves better in them than in water. In the present experiments three aniline dyes were used: methylene blue, neutral red, and Gentian violet (National Aniline and Chemical Co.). We may test first the solubility of the dyes in narcotics. The dyes were dissolved in distilled water (pH = 7.1). The solutions containing 0.0008 percent of the dyes (about 20 cc. of each solution) were shaken with 3 cc. of chloroform and ether (Merck, redistilled before the experiments) and left for some hours. After a complete separation of the narcotics from water the concentration of the dye in water and in the narcotics was determined colorimetrically. It was found that methylene blue is much more soluble in water than in chloroform and insoluble in ether. Neutral red is much more soluble in ether and especially in chloroform than in water. Gentian violet is much more soluble in chloroform than in water and insoluble in ether. Therefore, if the author's opinion about the cause of a decreasing effect of small concentrations of narcotics upon permeability is correct, we may expect that ether and chloroform will decrease the permeability for methylene blue, while they will increase it for neutral red. At the same time, chloroform will increase it for gentian violet, while ether will decrease it for the same dye.

In every experiment 40 leaves cut from the same branch of *Elodea* were put into a 0.0008 percent solution of one of three dyes in spring water (pH = 7.3, 0.002 percent Ca, 0.002 percent Cl) poured in eight specimen vials closed by corks (five leaves into each glass). The dye solutions contained different concentrations of ether or chloroform. In two hours the leaves were taken from the containers and the permeability of their protoplasm for the dyes was determined colorimetrically. Preliminary experiments made in the same manner as described in the author's previous paper (13) showed that neither the adsorption of the dyes on the cell walls nor the absorptive power of the cell sap is noticeably changed by narcotics taken in concentrations which are sufficient for narcosis but do not produce a gradual death (for instance, ½ to 2 percent ether) while their lethal concentrations (3 percent ether) slightly diminish the adsorption of the dyes on cell walls. As this adsorption (from 0.0008 percent dye solutions) gives only a small part of the tint of the leaves which have been immersed in 0.0008 percent dye solutions, one has to conclude that it did not influence the results given below. The possible influence of narcotics on the permeability of the cell walls to the dyes is not important in our case because the permeability of the cell walls in *Elodea* (a water plant) is many times greater than that of protoplasm, and the penetration of dissolved substances into

the cell is regulated almost exclusively by protoplasm. Thus the figures given below concern protoplasm.

In all experiments with the absorption of dyes the latter were dissolved, as in the author's previous experiments on the influence of light (13), in spring water the pH of which was 7.3. The variation of pH from 6 to 8 did not influence the final result, however.

In tables 1, 2, and 3 an average permeability for five leaves from each vial is given. It should be remembered that the permeability, expressed in grams of a dye going through one square centimeter of the protoplasm layer during one hour divided by the concentration of the dye in percent, is equal to about $5 \cdot T \cdot 10^{-5}$ in the case of methylene blue because $C/T = 37/10^6$ (13). The preliminary experiments showed that this ratio is equal to $39/10^6$ for gentian violet and to $150/10^6$ for neutral red. Therefore, the permeability for gentian violet may be considered equal to that of methylene blue ($5 \cdot T \cdot 10^{-5}$), while the permeability for neutral red is $20 \cdot T \cdot 10^{-5}$. T represents the tint of the scale leaves (expressed in minutes of their immersion in a 0.0025 percent solution of the dye) (13). Sunlight was diffuse and temperature 24° C.

TABLE 1. *Methylene Blue*

Concentration of ether in %*	0	0.5	1	1.5	2	2.5	3	3.5	4	
Permeability × 10^5	47	41	19	31	54	77	Dead in part	All cells are dead		
Protoplasmic movement	+	+	+	+	−	−	−			
Concentration of chloroform in %	0	0.02	0.05	0.07	0.1	0.125	0.15	0.2	0.25	0.3
Permeability × 10^5	53	50	35	37	40	55	60	95	114	112
Protoplasmic movement	+	+	+	+	+	+	−	All cells are dead		

* By percentage is understood grams of substances in 100 cc. of the solution.

TABLE 2. *Neutral Red*

Concentration of ether in %	0	0.5	1	1.5	2	2.5	3	3.5
Permeability × 10^5	150	174	208	260	250	264	Dead in part	All cells dead
Protoplasmic movement	+	+	+	+	−	−		
Concentration of chloroform in %	0	0.02	0.05	0.075	0.1	0.125	0.15	0.2
Permeability × 10^5	190	190	290	284	310	328	400	400
Protoplasmic movement	+	+	+	+	+	−	−	All cells dead

TABLE 3. *Gentian Violet*

Concentration of ether in %	0	0.5	1	1.5	2	2.5	3	3.5
Permeability × 10^5	26	26	18	24	34	36	36	*
Protoplasmic movement	+	+	+	−	−	All cells are dead		
Concentration of chloroform %	0	0.25	0.05	0.075	0.1	0.15	0.2	0.25
Permeability × 10^5	24	34	35	38	44	53	70	70
Protoplasmic movement	+	+	+	+	−	All cells are dead		

From tables 1 to 3 it may be concluded that only the solubility of a dye in a narcotic is important for the change of the permeability for this dye, produced by small concentrations of the same narcotic. Indeed, if the penetrating substance is more soluble in water than in a narcotic the permeability is decreased; on the contrary, if it is more soluble in the narcotic the permeability is increased. On the other hand, the increase of the permeability of protoplasm produced by narcotics taken in strong concentrations does not depend on the solubility at all; it results from a gradual dying of the cells due to the poisonous action of narcotics. Cell death, a decomposition of the principal chemical compounds of the whole mass of the living matter contained in the cell, is accompanied by a loss of the selective permeability of protoplasm. A partial decomposition of these compounds leads to an increase of the permeability to water-soluble substances. The products of the decomposition may coagulate or partly dissolve in the surrounding water. If the decomposition and the coagulation are only slight, a recovery of life is possible, but if this decomposition is marked, the increase of permeability produced by stronger concentrations of narcotics is irreversible. It is not necessary to discuss here other theories of the increase of permeability produced by narcotics because they were submitted to a sufficient criticism in Winterstein's book.

Mechanical Influences and Narcotics

The author's former experiments showed that different mechanical agents (as for instance, blows, pressure, or cutting) produce first a decrease of the resistance of living matter to harmful effects (high temperature, poisons, etc.), and then a complete decomposition of its principal compounds and coagulation (or a partial dissolution in water, or cytolysis)(8, 9, 10). In other words, the effect of mechanical agents is similar to that of stronger concentrations of narcotics. We may expect, therefore, that mechanical influences will change the permeability in a similar manner. Moreover, the author's experiments showed that mechanical effects can spread from cell to cell, and that weak effects which follow each other may add to each other. The same action may be also expected with permeability.

The experiments with the absorption of methylene blue by *Elodea* leaves showed first of all that the mechanical effect produced by cutting spreads demonstrably only to two layers of cells adjacent to the cut. If the leaves were cut into small pieces and immersed in a 0.0008 percent solution of methylene blue, an investigation under the microscope in two hours showed that the cell layers just mentioned had absorbed much more dye than the cells in the middle of the piece. Therefore, if the area of the pieces was about two to three square mm., their tint was distinctly more intensive than that of uninjured leaves immersed in the same dye solution.

In order to produce a mechanical effect on all cells of the leaf, 10 leaves were cut from the same spot of an *Elodea* branch, and five of them were

immersed for 10 minutes in a 35 percent solution of cane sugar where all cells were plasmolyzed and then put into water again. In five minutes all leaves were immersed in the solution of methylene blue (0.0008 percent). After two hours the permeability of protoplasm was determined colorimetrically. It was found to be, on the average, 32 in the normal leaves and 55 in the leaves which had been plasmolyzed. This experiment was repeated several times with a similar result.

The application of the method of isotonic coefficients gave similar results. The mechanical influence of cutting reinforced the effect of the slight plasmolysis, and a distinct increase of permeability was observed as may be seen from table 4, where C' represents the isotonic concentration of KNO_3, average for 8 leaves; C_s the isotonic concentration of sugar; C the concentration of the solution of KNO_3 which has the same osmotic pressure as the sugar solution of the concentration C_s (average from that calculated from freezing point and that from electrolytic dissociation); and μ is the factor of permeability calculated from the formula $\mu = 1 - \frac{C}{C'}$. These figures are given for leaves cut from the plant 20 hours before the experiment, for the same leaves but cut into small pieces (about 2 mm. wide) before the experiment, and for the same pieces left for 20 hours in water. All concentrations are given in gram-molecules per liter. Diffuse sunlight. Temperature 26° C.

TABLE 4. *Lower Cell Layer of Elodea Leaves*

Leaves Cut Off 20 Hours before the Experiment				Leaves Cut Into Pieces				Pieces after 20 Hours			
C'	C_s	C	μ	C'	C_s	C	μ	C'	C_s	C	μ
0.355	0.49	0.295	0.17	0.45	0.51	0.315	0.30	0.43	0.54	0.335	0.22

The corresponding experiment on *Tradescantia* was made in the following manner: One large piece of epidermis (14 mm. long, 4 mm. wide) was cut into two approximately equal pieces at 9:40 a.m. One of these pieces was cut once more into four pieces, and then all pieces were left in spring water. At 2:10 P.M. the other half of the large piece was cut into eight pieces, and the factor of permeability of all pieces was determined. The results are given in table 5. The experiment was made in diffuse sunlight, temperature 25° C. All concentrations are given in gram-molecules per one liter.

TABLE 5. *Epidermal Cells of Tradescantia*

Slices No.	Four Large Pieces Made 4½ Hours Before Investigation				Slices No.	Eight Small Pieces Immediately Investigated			
	C'	C_s	C	μ		C'	C_s	C	μ
1	0.144	0.216	0.122	0.16	1	0.151	0.216	0.122	0.20
2	0.143	0.214	0.121	0.16	2	0.151	0.218	0.123	0.19
3	0.144	0.216	0.122	0.16	3	0.153	0.222	0.125	0.19
4	0.141	0.210	0.119	0.17	4	0.153	0.222	0.125	0.19
					5	0.154	0.220	0.124	0.20
					6	0.155	0.222	0.125	0.20
					7	0.154	0.220	0.124	0.20
					8	0.154	0.218	0.123	0.21
	Average μ = 0.16					Average μ = 0.20			

As is known, if the slices of *Tradescantia* epidermis are too thin, the cells become colorless in a few hours. In this case the cells were evidently so strongly affected mechanically by cutting that they gradually died. But if the slices are of a proper thickness, at least the cells in the middle of a slice recover, and then the slices can be kept in water (pH = 7.3) for several days in good condition. The present experiments showed that the factor of permeability decreases with the recovery of the cells, but after the minimum permeability has been reached the permeability increases again a little. So, for instance, the isotonic concentrations of KNO_3 and sucrose found one hour after the preparation of 6 slices were at an average 0.141 and 0.212 (μ = 0.15), while these concentrations found 2½ hours after the slices had been cut from the same spot of the leaf were 0.131 and 0.208 (μ = 0.10). They were 0.122 and 0.202 (μ = 0.07) four hours after preparation of the slices. Left in water (pH = 7.3) for 18 hours the slices showed, however, a slight increase of the permeability again (the concentrations were 0.134 and 0.208, μ = 0.12). It is therefore very probable that the decrease of permeability observed by Fitting (1) in the plasmolysing solutions of KNO_3 was produced by a recovery of cells after the mechanical effect of slicing but not by the action of the salt, as Fitting thinks. In accord with the results of the present experiments Höfler (3), who could observe details of this process, came to the conclusion that permeability first increases and then decreases after several hours. In the process of slicing every cell is affected mechanically because a good slice contains only two or three layers of cells.

Not only a cutting of neighboring cells but also an energetic bending of the cells produces a mechanical effect which can be detected with the method of isotonic coefficients. A piece of epidermis of *Tradescantia* one and a half centimeters long was washed out in water and cut into two equal pieces, one of which was cut in four more pieces; and then all epidermis pieces were left in water for 18 hours. The next morning the other large piece was bent for at least 50 times, then cut into four pieces, and the permeability factors determined. The results are given in table 6.

TABLE 6. *Epidermal Cells of Tradescantia*

Slices No.	After 18 Hours in Water			μ	Slices No.	After the Mechanical Influence of Bending and Cutting			μ
	C'	C_s	C			C'	C_s	C	
1	0.124	0.206	0.116	0.065	5	0.133	0.208	0.118	0.113
2	0.124	0.208	0.118	0.049	6	0.133	0.208	0.118	0.113
3	0.130	0.210	0.119	0.050	7	0.134	0.208	0.118	0.120
4	0.128	0.209	0.118	0.049	8	0.134	0.208	0.118	0.120
		Average μ = 0.05					Average μ = 0.12		

In the author's previous paper concerning the protective action of narcotics on the resistance of protoplasm to poisons it was stated that

ether protects yeast cells from the injurious effect of chloroform (11). The present experiments show that chloroform in a sufficient but harmless concentration, that is, one producing a decrease of permeability, hinders the influence of cutting on permeability to some extent if the chloroform does not act on protoplasm too long. The results of the experiment, which was similar to that described above (table 5) but modified so that the cutting of the second half into eight small pieces was made in water containing 0.1 percent chloroform in which the slice had been for five minutes, are given in table 7.

TABLE 7. *Epidermal Cells of Tradescantia*

Slices No.	Four Large Slices Made 4½ Hours before the Investigation				Slices No.	Eight Small Slices Cut in Chloroform (0.1%) and Immediately Investigated			
	C'	C_s	C	μ		C'	C_s	C	μ
1	0.128	0.204	0.115	0.10	1	0.150	0.232	0.130	0.13
2	0.132	0.208	0.118	0.11	2	0.140	0.214	0.121	0.14
3	0.148	0.226	0.126	0.15	3	0.150	0.232	0.130	0.13
4	0.124	0.196	0.110	0.11	4	0.150	0.232	0.130	0.13
					5	0.146	0.226	0.127	0.13
					6	0.150	0.232	0.130	0.13
					7	0.140	0.214	0.127	0.14
	Average μ = 0.12					Average μ = 0.13			

LIGHT, NARCOTICS, AND THE PERMEABILITY OF PROTOPLASM

In a recent paper (13) the author showed that light decomposes some unknown substance (or substances) in protoplasm, which takes part in the production of its selective permeability. The decomposed substance is restored in the dark, and the permeability decreases again. The changes of the permeability were determined by the method of the absorption of methylene blue. Zycha (19), using the method of isotonic coefficients, could not detect any changes of permeability produced by light. In the present paper some new experiments are described in which the changes of permeability were observed by means of the method of isotonic coefficients, and the object used was the same as in Zycha's experiments: epidermal cells from the main vein of the lower side of the leaf of *Tradescantia* (*Rhoeo*) *discolor*.

One large piece of epidermis (12 mm. long, 4 mm. wide) was cut from the plant and immediately cut into eight pieces which were left in water till the next morning. At 10 A.M. four pieces of epidermis were transferred to a dark room where they remained till 12 P.M. Then the permeability factors of all pieces of epidermis were determined simultaneously. The results are given in table 8. The determination of the permeability factors of the epidermal pieces left in the dark room was made in the same room. A weak electric light (5 watt) filtered through a concentrated solution of potassium bichromate was used for illumination.

TABLE 8. *Epidermal Cells of Tradescantia*

Slices No.	Diffuse Sunlight				Slices No.	Dark Room			
	C'	C_s	C	μ		C'	C_s	C	μ
1	0.130	0.200	0.113	0.14	5	0.130	0.210	0.119	0.09
2	0.132	0.204	0.115	0.13	6	0.130	0.209	0.118	0.10
3	0.132	0.208	0.118	0.11	7	0.128	0.210	0.119	0.07
4	0.132	0.206	0.116	0.12	8	0.128	0.208	0.118	0.08
		Average μ = 0.12					Average μ = 0.08		

This experiment was repeated several times with the same result. Always the permeability factors were found greater in light than in the dark.

As was mentioned above, the method of isotonic coefficients was used by Zycha but in an erroneous manner. His experiments also differed so much from those of the author that it would not be surprising if he could not obtain the same results even if he used the above method correctly. He put the whole plant into the dark, while in the author's experiments only slices of the epidermis were kept in the dark. It is therefore quite impossible to assert that Zycha studied the influence of light upon the permeability of epidermal cells which before they are cut are influenced by the neighboring tissues of the plant. *Tradescantia discolor* is a succulent; it may therefore form organic acids in its tissues if put into the dark. Acids increase the permeability of protoplasm. Thus, it is impossible to say at present what influences produced changes of permeability in a few of his experiments where the difference between the osmotic coefficients is too great to be ascribed only to his erroneous use of the method of isotonic coefficients. On the other hand, the author has shown that the maximum permeability is reached if the illumination is about 0.1 of the direct sunlight. If the illumination is increased, no further increase of permeability, or even gradual decrease, is observed. This may explain why Zycha's plants which had been illuminated for several hours by direct sunlight showed a smaller permeability (Zycha, l.c., p. 519) than the plants which were investigated during overcast weather, although this difference in permeability found by Zycha could be also produced by the formation of acids or other unknown factors acting from the side of neighboring tissues.

The experiments concerning the influence of ether and chloroform upon the changes of permeability produced by light were made as follows

Elodea leaves were immersed in solutions of methylene blue (0.0008 percent) containing ether or chloroform and in control solutions without narcotics. Then they were exposed to different conditions of illumination. If the leaves were exposed to direct sunlight, the solutions had to be changed every fifteen minutes to prevent the fading of the dye, and the containers were immersed in a water bath (temperature 26° C.). The permeabilities found are given in table 9.

From these results it may be concluded that although narcotics decrease

TABLE 9. *Elodea Leaves Immersed in a 0.0008 Percent Solution of Methylene Blue for Two Hours*

	Diffuse Sunlight		Dark		Direct Sunlight	
Concentration of ether in %.....	0	1	0	1	0	1
Permeability.....	80	30	40	20	115	71
Concentration of chloroform in %...	0	0.05	0	0.05	0	0.05
Permeability.....	75	65	37	33	118	89

the permeability in all conditions of illumination, they do not prevent the changes of the permeability produced by changes of illumination. Even an increase of the concentration of ether to two percent which usually increases permeability does not prevent the changes of the permeability by light, as is seen from the following figures.

Ether Concentration	Diffuse Sunlight Permeability	Dark Permeability	Direct Sunlight Permeability
0.....	58	33.....	98
2%.....	65	35.....	95

A further increase of the concentration of the narcotic is harmful and finally leads to death. It is therefore not surprising that such concentrations which gradually destroy the whole colloidal system of protoplasm stop the changes of the permeability.

These results confirm the supposition that the increase of permeability produced by light and that produced by mechanical agents differ in their nature.

SUMMARY

1. Small concentrations of narcotics (ether 0.5 to 1.5 percent, chloroform 0.02 to 0.1 percent) decrease the permeability of protoplasm for water-soluble substances if these substances are insoluble or less soluble in the narcotics used than in water. However, if these substances are more soluble in the same narcotics than in water, the permeability of protoplasm is increased by the narcotics.

2. The determination of the permeability was made by the method of the absorption of aniline dyes by the leaves of *Elodea canadensis*, using dyes of different solubility in water and in the narcotics. The dyes were methylene blue, neutral red, and gentian violet.

3. Concentrations of ether above 1.5 percent and of chloroform above 0.1 percent increase the permeability of protoplasm. This increase is at first reversible, but becomes irreversible if the concentrations increase. A further increase of the concentration leads to death. The explanation of the process is given in the text.

4. Mechanical agents, as for instance cutting, bending, plasmolysis, and deplasmolysis, produce an increase of the permeability of protoplasm of the affected cells. This effect spreads to the neighboring cells but very soon

decreases in strength. In a few hours the increase is transformed into a slight decrease of the permeability which in 12 to 18 hours becomes normal.

5. Narcotics prevent the increase of the permeability produced by mechanical agents, but do not prevent its increase produced by light, these processes being different in their nature. Only the concentrations of narcotics which gradually kill the cells stop the changes of permeability produced by light.

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PENICULUS, A NEW GRASS GENUS FROM BRITISH HONDURAS

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A species of a previously unknown genus of grasses was collected in British Honduras in February, 1931, on an expedition of the Herbarium and Museum of Zoölogy, University of Michigan, collaborating with the Department of Historical Research, Carnegie Institution of Washington, in a biological survey of the Maya area. The name *Peniculus* has reference to the stiff hairs on the glumes and sterile lemma which makes them appear like tiny brushes.

Peniculus Swallen, gen. nov.

Spiculae tuberculato-hispidae uniflorae infra glumas articulatae, lemmate sterili infra lemma fertile donatae; glumae subaequales, prima 3-nervi, secunda 5-nervi; lemma fertile firmum marginibus tenuibus; palea non carinata, lemma fertile aequans; stamina 3; stigmata plumosa.

Gramen perenne dense caespitosum, culme gracili, racemis solitariis angustis.

Spikelets tuberculate-hispid, alternate on opposite sides of the rectangular rachis, one-flowered with a sterile lemma similar to the glumes below the fertile one, articulated below the glumes and falling entire from the very short crateriform pedicels; glumes rounded on the back, the first 3-nerved, the second 5-nerved, a little longer than the first; sterile lemma 5-nerved, equaling the second glume; fertile lemma rounded on the back, equaling the second glume and sterile lemma, firm, membranaceous, 5-nerved, the nerves obscure, the margins thin, not inrolled, inclosing the palea; palea thin, nerveless or nearly so, not keeled, a little shorter than the lemma; stamens 3; stigmas plumose.

Densely tufted perennial grass with erect slender culms bearing single spikelike racemes.

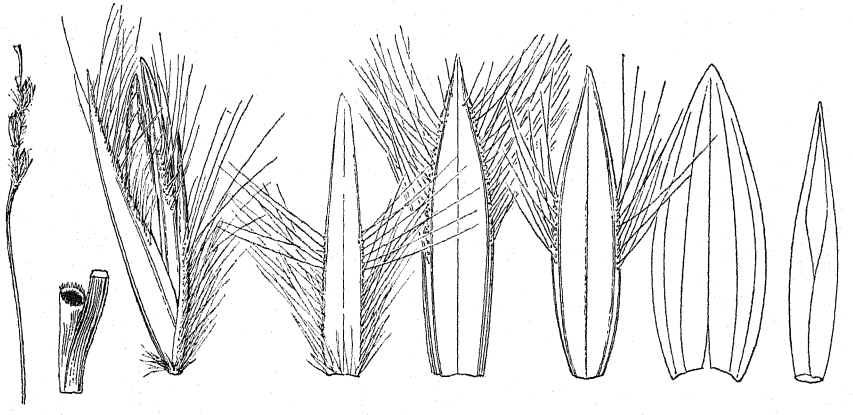
Type species *Peniculus angustifolius* Swallen

Peniculus angustifolius Swallen, sp. nov. (text fig. 1)

Culmi dense caespitosi graciles erecti 25–40 cm. alti laeves glabri, nodis pubescentibus; vaginae laeves, glabrae vel infimae pubescentes; laminae involutae usque ad 30 cm. longae, 1 mm. latae, laeves, glabrae; ligula ciliata 0.5 mm. longa; racemi 2–5 cm. longi terminales solitarii; spiculae appressae 5 mm. longae; pedicelli 1 mm. longi pubescentes apice crassi, crateriforme; gluma prima acuta 4.5 mm. longa, nervis lateralibus infra tuberculato-ciliatis, pilis usque ad 3 mm. longis; gluma secunda acuta 5 mm. longa, inter nervis lateralibus supra ciliatis; lemma sterile 5 mm. longum, inter nervis lateralibus infra ciliatis; lemma fertile 4.5 mm. longum firmum

glabrum; palea enervis, quam lemma fertile paulo brevior; antherae 2 mm. longae.

Culms densely tufted, slender, erect, 25–40 cm. tall, smooth, glabrous, the nodes pubescent; sheaths smooth, glabrous or the lowermost appressed-pubescent; blades firm, the lower ones thickened toward the base, involute, those of the main culm 5–9 cm. long, the uppermost much reduced, those of the innovations elongate, as much as 30 cm. long, about 1 mm. wide unfolded, smooth, glabrous except for a few hairs near the base on the lower



TEXT FIG. 1. *Peniculus angustifolius*. Left to right: Raceme, × 4/5; part of rachis showing pedicel, × 8; spikelet, × 8; first glume, × 8; second glume, × 8; sterile lemma, × 8; fertile lemma, × 8; palea, × 8.

side; ligule ciliate, less than 0.5 mm. long; raceme solitary, terminal, 2–5 cm. long, the rachis flexuous, rectangular in cross section; spikelets 5 mm. long, appressed to the rachis; pedicels 1 mm. long, pubescent, thickened above, the margins of the crateriform summit short-ciliate; first glume 4.5 mm. long, acute, the midnerve obscure, the lateral ones prominent, stiffly tuberculate-ciliate on the lower half, the hairs ascending except those at the middle, these stiffly spreading, about 3 mm. long; second glume 5 mm. long, acute, the lateral nerves approximate, not more prominent than the midnerve, the internerves strongly tuberculate-hispid on the upper half, the hairs stiffly spreading, turned inward toward the fertile floret; sterile lemma 5 mm. long, the lateral nerves more prominent, the internerves tuberculate-hispid above the middle with ascending hairs, the summit naked; fertile lemma 4.5 mm. long, firm, acute, glabrous, the margins inclosing the palea; palea acute, a little shorter than the lemma, not keeled, nerveless; anthers 2 mm. long.

Type in the U. S. National Herbarium no. 1535396 collected on Mountain Pine Ridge, El Cayo District, British Honduras, February 25, 1931 by H. H. Bartlett (no. 11748).

Peniculus is most nearly related to the Australian genus *Neurachne* R. Br. of the tribe Zoysieae. The spikelets of these genera are similar in having a sterile lemma below the fertile floret, but the glumes and sterile lemma of *Peniculus* are less indurate and less strongly nerved than in *Neurachne*

while the fertile lemma of *Peniculus* is more indurate than that of *Neurachne*. The inflorescence of *Peniculus* is a few flowered two-sided raceme, that of *Neurachne* is a densely flowered spiral raceme or spike. The blades of *Neurachne* are short and flat and those of *Peniculus* are involute and, except for the uppermost ones, elongate.

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THE DEVELOPMENT OF THE SEXUAL ORGANS IN *LEPTOLEGNIA CAUDATA*

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Leptolegnia is one of the less common genera of the Saprolegniaceae and until recently has been represented by only one species, *L. caudata* deBary. This species was first tentatively placed in *Saprolegnia* by deBary (12), but later (13) he established the genus *Leptolegnia* based on the one species. The mycelium is typically composed of a circular, whitish fringe of regular threads around a fly or other insect or bit of seed in water. The strains vary considerably in their robustness, the form from Chapel Hill being considerably less robust than those from Long Island or Florida, the threads of the latter often approaching a well developed *Saprolegnia* in thickness and length. The sporangia, in their thread-like character, resemble those of *Aphanomyces*, though the behavior of the spores in *Leptolegnia* is more like that of the spores in *Saprolegnia*. The oögonium in *Leptolegnia* shows a superficial resemblance to that of *Aphanomyces* in that each contains only one egg. The beak-like process on the oögonium, which usually grows out where the antheridia are applied, the exceedingly thick oöspore wall, the peculiar eccentric arrangement of the oil globules in the ripe oöspore, and the fact that the oöspore completely fills the oögonium are characters which make this genus stand out from the other genera of the *Saprolegniaceae*. The ripe oöspore of *Pythiopsis cymosa*, however, with its lunate cap of oil globules is quite similar to the oöspore of *Leptolegnia*.

Although the fungus has been reported from a number of diverse regions its occurrence is rather infrequent. In Europe it has been reported from Germany by deBary (12, 13), Fischer (14) and von Minden (18); from Denmark by Müller (21) and Petersen (25); and from Latvia by Apinis (1). In America it was first reported from North Carolina by Coker (7) and has since been collected by Thaxter in New England, by Pieters at Ann Arbor, Michigan, in Florida, and on Long Island, N. Y. (see Coker, 8). It is interesting to note that Petersen (25, p. 511) and Müller (21) record the fungus as an exceedingly destructive parasite on *Leptodora Kindtii*.

THE PROBLEM

Leptolegnia caudata is particularly interesting to the student of sexuality in the *Saprolegniaceae* since it is the only species of the family in which deBary (12) ever seriously suspected that fertilization might take place. In spite of the fact that one of his figures (13, fig. 5, d) illustrates an unmistakable stage in the passage of material from the antheridium into the oögonium, he finally stated (13, p. 632) that he was unable to detect the passage of any substance from the male into the female organ. Although von Minden

(18) has nothing to say in regard to fertilization his figure 4*b* strongly suggests that such an act occurs. Both oögonia in this figure show a receptive spot and one of the antheridia is nearly completely empty. In regard to fertilization, Coker (8, p. 159) states: "Two, three, or even more antheridia to the oögonium were common in our material. . . . The antheridium is full of protoplasm when it is cut off, and is empty a little later; and the amount of protoplasm contained in it is so large that a discharge into the oögonium seems probable. When the empty antheridium is pulled from the oögonium, a distinct circular opening can be seen in it, and the opening in the original membrane on the oögonium can be easily made out (fig. 4)." So far as I have been able to find, these are all the observations on the development of the sexual organs in *Leptolegnia*.

Much difficulty was at first experienced in getting large enough quantities of the sexual organs of *Leptolegnia* to carry out the cytological studies. A very small percentage of the cultures made on various seeds and insects in water produce sexual organs. When the sexual organs do appear, instead of being evenly distributed over the entire culture they often begin to form in several small clusters. These clusters increase in size, and usually, even when the cultures are fully mature, the clustered habit is still apparent. Other observers, Fischer (14, p. 386), Petersen (25), Thaxter (in a letter to Dr. Coker), Pieters (see Coker, 8), and Coker (8) either report the fungus as being sexually sterile or as forming sexual bodies very rarely. It was necessary, therefore, first of all to discover the conditions which favored the production of sexual organs.

Sexual Nature of the Thallus

Several peculiarities of the fungus seemed to point to a heterothallic condition of the mycelia. The sexual organs always appear in ordinary cultures on hemp seed or termites with great irregularity and instead of being scattered evenly over the entire culture are almost always grouped in clusters as though the two types of mycelia were present only in such clusters. The oögonia and antheridia, moreover, appear always to arise from separate threads. To determine the sexual nature of the thallus, single spore cultures were made. In a small percentage of the cultures descended from each of the single spore strains oögonia appeared. Numerous attempts to cross strains collected from different localities (Cold Spring Harbor, L. I., N. Y.; Chapel Hill, N. C.; Madison, Wis.) failed to indicate any sexual dimorphism in the species. It was concluded, therefore, that these strains of *Leptolegnia* were homothallic.

Influence of External Conditions on Sexual Reproduction

Temperature

The results of Coker (7) suggested that cold might stimulate the formation of sexual organs. After his plant was cultivated for three months with-

out the appearance of sex organs, they finally appeared during the Christmas recess while there was no heat in the building.

To test the influence of cold, cultures on hemp seed and termites have been kept in a temperature of 14° C. in a cold room rather poorly illuminated by a small window. The rate of sexual reproduction was considerably retarded by low temperature, but no appreciable difference in the number of oögonia and antheridia finally formed could be observed in such cultures as compared with those kept in room temperature (about 18°–22° C.).[1]

High temperatures (27°–35° C.) seem to inhibit completely the formation of sexual reproductive organs. Cultures kept in an incubator with the temperature about 27° C. and others kept in room temperature during very warm summer weather (22°–37° C.) failed to reproduce sexually. The remarkable inhibitory influence of high temperature on sexual reproduction was best shown in a series of experiments in which the fungus was grown on the media which had been found (see below) to produce the largest number of sexual organs at room temperature (18°–22° C.), but in these experiments the cultures were kept in an incubator with the temperature about 27° C. In such experiments no sexual organs were formed. The fungus is a very rapid grower at temperatures ranging from 25°–30° C. whereas at temperatures below 18° C. growth is slowed down as the temperature is lowered. Sexual reproduction seems in some way to be connected with the checking of growth by low temperatures.

The influence of temperature on sexual reproduction in *Leptolegnia* is particularly interesting because in certain other species of water fungi as *Achlya orion* Coker and Couch (see Coker 8, p. 114) temperature has only a slight influence on sexual reproduction, the fungus apparently being capable of reproducing sexually under any temperature which produces vigorous healthy growth.

Light

Light does not appear to have any influence on sexual reproduction in *Leptolegnia*. Cultures kept in the refrigerator (14°–18° C.) in the dark produced abundant sexual fruits. Light does have such an influence in certain other water fungi, *e.g.*, in *Aphanomyces exoparasiticus* (Couch, 10). In this species when the fungus is grown on corn meal agar on room tables and thus subjected to the alternation of daylight and darkness the oögonia are formed in circular areas, a new circle of oögonia appearing every day.

[1] The strain of *L. caudata* used in all my experimental and cytological work described here was collected at Cold Spring Harbor, L.I., N.Y. During the summer of 1931 I carried on a series of experiments to test the influence of temperature on the formation of sexual organs in a strain of *L. caudata* collected near Raleigh, N. C. Twenty cultures were made on bits of boiled hemp seed in autoclaved distilled water treated with animal charcoal. Ten were put in a refrigerator where the temperature varied from 16°–20° C. and the other ten were kept at room temperature, 20°–30° C. All the cultures in the refrigerator formed sexual organs, while none of those in the room produced any. The experiment was repeated several times during the summer with the same results.

If such cultures are kept in total darkness, no sexual reproduction occurs. Just how much light was necessary for sexual reproduction was not determined with *A. exoparasiticus*.

Nutrients

To increase sexual reproduction the following solutions (all 1/100 molar solutions) of salts were added to young cultures on hemp seed: Calcium nitrate, potassium sulfate, potassium nitrate, potassium phosphate, and sodium phosphate. Good growth was obtained in all these solutions but the number of cultures bearing sexual organs was not appreciably increased.

Cultures on hemp seed to which were added solutions of *M*/200 levulose, 1% asparagin, 1% and 0.5% hemoglobin, and 0.5% meat peptone failed to show any increase in the number of sexual organs.

Various combinations of salts, sugars, and proteins have been tried and one combination has been found in which sexual reproduction occurs abundantly and regularly in all cultures. Cultures were made on hemp seed and put in a solution containing 0.5% maltose, 0.5% malt extract, and 0.1% meat peptone. After three days a dense mat of mycelium was formed, but no reproduction either asexual or sexual had occurred. The solutions were replaced by sterile well water, and two days later a thin growth of mycelial threads had grown out from the thick region. Many sexual bodies were forming on the dense mat of mycelium especially in the region next to the renewed growth. Subsequent experiments have shown that the maltose or malt extract, or even the hemp seed may be left out without diminishing the number of oögonia.

Interesting results were obtained by measuring the pH of the above solutions after cultures of the fungus had been growing in them for 4 or 5 days. The initial pH of the solution after sterilization was 4.6, but this was lowered by the growth of the fungus to 3.4–3.2.

Hydrogen-ion Concentration

Some preliminary work has been done to test the effect of the hydrogen-ion concentration on the growth and reproduction of the fungus. In the first experiments eight of the buffer solutions described by Clark and Lubs (5) with pH values from 4 to 11 were selected. Cultures of the fungus on hemp seed in artesian well water (with pH about 7) were made and when the growth had started well, the water was replaced by the buffer solutions. All of the solutions, however, were decidedly toxic to the fungus.

Solutions were then made with artesian well water and HCl and Na_2CO_3 in such proportions as to give approximate pH values from below 3 to 9.8 +. Eleven HCl solutions were made and six with Na_2CO_3. The approximate pH values of these fluids were tested by the colorimetric method as described in Clark (4). The acid solutions were designated 1 to 11, solution 1 being made according to the directions given in Clark (4, p. 39) except that artesian well water with a pH value slightly below 7 was

used instead of distilled water. This solution (1) had a pH value about 5, and the other acid solutions 2 to 11 had pH values 4.8, 4.6, 4.4, 4.2, 3.8, 3.6, 3.5, 3, 2.6, respectively. The alkaline solutions were designated 1 to 6 and had pH values from slightly above 7 to slightly over 9.8, respectively. The results of these experiments may be summarized as follows: Growth was excellent in all solutions from pH about 9 down to pH about 3. In solution 11 five times as much acid was added as in solution 10, the resulting mixture having a pH of about 2.6. Growth in this was very poor, not visible to the unaided eye, only a few, delicate, branched threads being visible under the low power of the microscope. No sporangia formed in any of the cultures. In both solutions with pH 5 and pH 7 a considerable number of oögonia were formed in some of the cultures and in one of the cultures in a solution with pH about 4.2 a few oögonia were formed. In none of the other solutions were any oögonia formed. In all the solutions in which the fungus grew well, both acid and alkaline, the pH value was changed by the fungus back to approximate neutrality, this change occurring during the first twenty-four hours after the fungus was in the solution.

Interesting results were obtained by growing the fungus on a series of solid nutrient media with different hydrogen-ion concentrations. The culture medium was prepared and titrated as follows: To 2000 cc. of the nutrient solution (maltose 0.5%; meat peptone 0.1%) 40 grams of agar was added. This was heated enough in an autoclave to melt the agar and then divided into ten 200 cc. portions in small, clean, pyrex flasks and then autoclaved to 15 lbs. pressure for 10 minutes. The flasks were numbered 1–10 and the varying amounts of sterilized, one-tenth normal H_3PO_4, as shown in the accompanying table, were added to the several flasks. The pH values

TABLE 1. *Experiments to Test the Influence of Varying the pH on Growth and Reproduction in Leptolegnia. Second Series, Feb. 10, 1927. At Room Temperature, 16–21° C.*

No.	Initial pH	Amt. in cc. $\frac{N}{10}$ H_3PO_4 to 200 cc.	Average Diameter of Colonies after: 96 Hours	168 Hours	Sexual Reproduction
1	5.8	0.1	86.5 mm.	90 mm. covered dish	No sexual reproduction
2	5.5	0.2	85 "	90 mm. covered dish	No sexual reproduction
3	5.2	0.4	71.5 "	90 mm. covered dish	No sexual reproduction
4	5.0	0.6	52.5	90 mm. covered dish	A few oögonia and antheridia formed
5	4.6	1.2	41.5 "	78.5 mm.	Many oögonia and antheridia formed
6	4.5	1.4	43 "	87.5 "	Many oögonia and antheridia formed
7	4.4	1.6	42 "	83.5 "	A few oögonia and antheridia formed
8	4.3	2	37 "	74 "	No sexual reproduction
9	4.1	3	24.5 "	51 "	No sexual reproduction
10	3.8	4	16 "	37 "	No sexual reproduction
Control	5.8	No acid added		Covered dish	No sexual reproduction

of the various fluids tested by the colorimetric method are also shown in the table. The culture fluid was then poured into Petri dishes, each 200 cc. being divided into about eight equal portions, i.e. about 25 cc. to each Petri dish. During the course of the experiments it was found that after the addition of the acid the pH value might be changed by sterilization in the autoclave. To avoid the necessity of more than one sterilization all of the above operations were carried out in a culture chamber. Before using the culture chamber it was thoroughly cleaned, the floor, walls, and ceiling being mopped with alcohol and then as an added precaution the chamber was closed up tightly and the walls steamed down. The most likely agents of infection are the spores of *Aspergillus*, *Penicillium*, and *Rhizopus;* but by using the culture chamber, and by keeping the laboratory clean and free from cultures of the above mentioned molds, it was possible almost entirely to avoid any infection of the plates.

The plates were inoculated by cutting out little agar squares from a plate on which the fungus was growing and placing a square on the edge of each plate. The experiments were run in duplicate. All the cultures were kept at laboratory temperature (16–21° C.) and were subject to the light changes of night and day. The diameter of the colonies in mm. was recorded every 24 hours. The cultures with the low hydrogen-ion concentration showed the most rapid growth; in fact the control cultures to which no phosphoric acid had been added showed the most rapid growth of any but the mycelium was not nearly so dense as in the acidulated solutions. The growth gradually decreased as the H-ion concentration increased until in the culture plates with a pH about 4.6 there was a sharp, decided decrease in growth while in the cultures with pH 4.5 and 4.4 the growth came back to almost the same strength as in the culture with a pH value of 5.0.

In the cultures with pH slightly above and those slightly below as well as in the one which showed the most decided falling off in growth there was considerable sexual reproduction. There was none in the control and none in the cultures with pH 5.8, 5.6, and 5.2.

The same results occurred in another set of cultures made at the same time and the experiments have since been twice repeated in duplicate with similar results.

Another set of cultures (in duplicate), made precisely as those above, were kept in an incubator with the temperature 27° C., the source of heat being a 25-watt carbon bulb. The light was partially covered by a black cardboard so that the cultures were practically in darkness even when the light was on. The growth results in these cultures were about the same as those kept out in the laboratory but no sexual reproductive organs were formed.

From the foregoing experiments it is obvious that a number of factors may influence the formation of sexual organs in *L. caudata*. It was found most convenient to induce their formation by growing the fungus on hemp

seed in maltose 0.5%, peptone 0.1%, solution at a temperature of about 18°–20° C. for two to three days and then replacing the solution with pure water. Such treatment, as a rule, induced the formation of large numbers of sexual organs.

Development of the Sexual Organs

Observations on Living Material

One can recognize that sexual reproduction is about to occur in *Leptolegnia* by the presence of numerous, rather delicate threads twining around the larger ones (Pl. XLII, fig. 1). The delicate threads give rise to the antheridia, while the larger threads bear the oögonia. The oögonia initials appear first and are indistinguishable in the early stages from those of *Achlya* and *Saprolegnia* in that they contain a large central vacuole enclosed by a dense layer of protoplasm, the vacuole extending back into the oögonial stalk. The basal wall separating the oögonium from its stalk may be laid down before the antheridia have reached the oögonium. By the time the basal wall is laid down in the oögonium, the central vacuole has disappeared. Several antheridia may be applied to each oögonium, but apparently only one ever functions. Wherever the antheridia are applied, the oögonium, as a rule, swells out into a beak-like protuberance at that spot so that the oögonium, spherical at first, may become quite irregular in shape. This protuberance may be called the receptive papilla since it is at this spot, in most cases, that the gonoplasm passes into the egg. The antheridia enlarge, becoming densely filled with protoplasm and tuberous in shape. Slightly over three days elapse between the appearance of the oögonial initials and the final maturation of the egg and therefore to attempt to get a complete series of uninterrupted observations on a single oögonium was impractical. The account here given is based on several series of interrupted observations.

Shortly after the receptive papilla has been formed the antheridial and oögonial walls at this point become thickened and hyaline, a process of hydrolysis apparently occurring (Pl. XLII, figs. 6, 7). While these events are taking place, the contents of the antheridium become arranged in a denser central mass and a lighter peripheral mass. After the walls between the oögonium and antheridium have been sufficiently softened, a very inconspicuous rift appears to extend from the antheridium down into the contents of the oögonium reaching almost to the center (figs. 7–10). Simultaneously with the appearance of the rift the denser central part of the contents of the antheridium slowly passes into the oögonium. The passage of this material is so slow as to be almost imperceptible. One can notice distinctly, however, that the antheridium is becoming partly empty of its contents (figs. 7–10). The functional antheridium never discharges all of its contents into the oögonium, but usually enough is discharged so that a conspicuous empty space is visible in the central part of the antheridium.

The non-functional antheridia retain their contents which eventually degenerate.

During the ripening of the egg it becomes invested with a very thick, double-layered wall and the oil content collects into many small droplets which form a sort of cup around half of the protoplasmic mass. Although my figure 13 shows the oil globules opposite the functional antheridium, they may occupy any position, showing, however, always the same eccentric arrangement in regard to the cytoplasm.

Observations on Fixed Material

As a killing and fixing fluid chrom-acetic acid proved the most satisfactory of several tried. It was necessary, however, to experiment considerably to determine the strength of solution, for it was found that one strength may give excellent results on one species and poor results on another. Furthermore, a solution may cause the collapse of the hyphae but fix the oögonia and antheridia in almost perfect condition. For example: Chamberlain's solution for marine algae caused most of the hyphae to burst at the tips with a consequent extrusion of the protoplasm. Flemming's medium without the osmic acid gave good results on hyphae but caused considerable distortion in the oögonia. Claussen's (6) solution gave only fair results with hyphae, causing considerable collapse of their contents, but was excellent for oögonia and antheridia. This solution was used most extensively. Material was fixed in it from 6–24 hours. In staining, Gram's method gave the clearest mitotic figures, while Heidenhain's iron alum haematoxylin gave the clearest views of extra-nuclear structures. Material was killed at various times during the night and day. By far the greatest number of mitotic figures was found in material killed after midnight (between 1 and 3 A.M.).[2]

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[2] Fixing and Staining Methods.

1. Have excellent material free from bacteria.
2. Kill material when the oögonia are in the stages desired: dividing nuclei are likely to be most numerous about midnight or a few hours thereafter; fertilization stages will probably be most numerous in the afternoon. Kill in Claussen's fluid (chromic acid 0.5%, acetic acid 1%) 6–24 hours.
3. Wash in still water, changing often, about 24 hours or until the material is thoroughly free from color.
4. Alcohol 15, 25, 35, 50, 60, 70, 80, 95, 100%, 2 hours each. While in 95% alcohol cut the material from the hemp seed or corn since these become very hard in alcohol and will dull the knife.
5. 100% alcohol ⅔, xylol ⅓; 100% alcohol ½, xylol ½; 100% alcohol ⅓, xylol ⅔; xylol; all 1 hour each.
6. Xylol + small lump of hard (56°) paraffin. Put in oven at temperature 30° C. 1 hour; slowly raise temperature and add more 56° paraffin, until temperature slightly exceeds 56° (i.e., until 56° paraffin remains melted). This process should take from 30 minutes to 1 hour, not more. Transfer to fresh melted 56° paraffin, 2 hours. Imbed in 56° paraffin, in perfectly clean watch glasses greased with glycerine.
7. Sections 5 μ thick.

The young oögonia and antheridia are multinucleate. The oögonium of average size contains about 30–40 nuclei; the antheridium about 6. These increase greatly in size and then undergo one mitotic division (Pl. XLIII, figs. 15, 17). In the early stages of mitosis the nucleus contains a very large, deeply staining granule and a spireme to which several smaller chromatin masses are attached (fig. 14). The nucleolus does not appear to be thrown out into the cytoplasm, but rather seems to be used in the formation of chromosomes. I was never able to be positive about the number of chromosomes, but during certain stages of mitosis they stand out with considerable individual distinctness and the number appears to be about eight.

Immediately following this mitosis all the nuclei in the oögonium except one migrate to the peripheral region and there degenerate. This appears to be the usual course of events, but I have found a number of oögonia in which the migration to the periphery and the selection of the egg nucleus preceded mitosis. The nucleus which remains in the center or which migrates to that position enlarges still more, becoming the female gamete nucleus. Sections of oögonia with a single nucleus or two which had been formed by division at or near the center and numerous nuclei in the peripheral region were quite common (fig. 18).

At about this stage of development I have found a very peculiar (probably abnormal) condition in a considerable number of oögonia, where a large number of the nuclei become arranged in a more or less hollow sphere.

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8. Place sections on slides smeared with thin coating of albumin fixative. Add one or more drops of water with pipette until sections float. Place slides in oven with temperature 30–35° (not higher, since sections do not stick if paraffin melts). As soon as ribbons have become smooth, drain off water with filter paper and then replace slides in oven until they are thoroughly dry.
9. Xylol, 5 minutes.
10. Absolute alcohol, 5 minutes.
11. 95%, 80%, 70%, 50%, 30% alcohol, 1 minute each.
12. Water, 2 minutes.
13. 1% aniline solution of Gentian Violet. (To make aniline solution of Gentian Violet: 2 cc. aniline oil + 18 cc. 95% alc., 80 cc. water. Now add 1 gram Gentian Violet.) 5–10 minutes in stain.
14. Water, 1 minute (just long enough to wash off the superfluous stain).
15. Iodine solution: water 300 cc., iodine 1 gram, potassium-iodid 2 grams; 3–5 minutes (1 minute would probably be enough and 5 minutes perhaps not too much). Solution should be kept in dark.
16. Water, 1–5 minutes, longer if necessary.
17. 95% alcohol, 30 seconds to 1 minute. Gentian Violet is very soluble in alcohol.
18. 100% alcohol, 2–3 minutes. (Too long in the absolute will dissolve out too much of the stain.)
19. Clove oil, 1–3 minutes. (Gentian Violet is also soluble in clove oil, but the stain dissolves more readily from the cytoplasm than from the nucleus.)
20. Cedar oil, 1 minute. Stops action of clove oil.
21. Xylol, 3–5 minutes.
22. Mount in balsam.

This sphere is often almost one-third the diameter of the oögonium and may contain twelve to eighteen nuclei. Just how and why this structure is formed I do not know. The structure suggests in a remote way the zonation of the nuclei in *Cystopus*.

The nuclei in the antheridium also undergo one mitosis. One of the daughter nuclei takes up a central position in the antheridium, enlarging considerably to become the functional male gamete nucleus (figs. 15, 18). In observations on living material in this stage of development one can easily recognize the denser protoplasmic mass in the center and the thinner, peripheral protoplasmic area.

At this stage several of the nuclei in the oögonium may show fairly distinct astral rays. On the female gamete nucleus these rays become very distinct. Stained with gentian violet they appear to be straight, extending as lines out into the cytoplasm (Pls. XLIII and XLIV, figs. 15, 17, 18, 20, 21, 22, 23), whereas with iron-alum haematoxylin the lines extend out straight for some distance and then appear to branch out into an elaborate network throughout the adjacent cytoplasm (fig. 19). A similar structure has been described by Miss Carlson (3) in *Achlya racemosa*. The cytoplasm surrounding the egg nucleus is finely granular while beyond this region the granules are coarser and the cytoplasm on the margin retains its vacuolated structure.

In this condition of development the oögonial contents as shown by gentian violet are differentiated, though sometimes vaguely, into a central uninucleated, denser, finely granulated region and a multinucleated, vacuolated, coarsely granular peripheral region. The antheridium also shows a distinct central gonoplasm and a peripheral vacuolated plasm (fig. 18).

The iron-alum haematoxylin staining showed a number of unmistakable cases of the hydrolysis of the membrane which separates the oögonium from the antheridium (figs. 16, 19). The gentian violet stain, however, did not bring this out. In the early stages of hydrolysis a number of minute granules appear on both sides of this membrane. These granules are not visible in the living material and are doubtless products of the fixation and staining methods, but are nevertheless indicative of a process taking place around the membrane. There are also a number of minute vacuoles next the membrane on the oögonial side. There are also vacuoles on the antheridial side of the membrane, but these are much larger. As the process of hydrolysis continues, the membrane becomes greatly thickened below the antheridium, appearing pale brownish with the iron-alum haematoxylin staining. The membrane appears to become so much softened that the greater pressure in the antheridium causes the membrane to bulge into the oögonium. As the pressure increases, the membrane gives way, and the antheridium discharges into the oögonium a single nucleus with some cytoplasm, which passes toward the egg nucleus leaving a path in the cytoplasm through which it has passed (Pls. XLIII and XLIV, figs. 19–23).

A somewhat similar condition has been observed by Cooper (9) in *Brevilegnia diclina* Harvey. From certain stained preparations one gets the impression that a considerable amount of vacuolar material passes over into the oögonium with the male nucleus (figs. 19–21). A few oögonia were seen in which the path made by or for the male nucleus had healed up and a wall had been laid down by the cytoplasm of the oöspore. This wall was thin compared to the oöspore wall and could be clearly seen to be connected with it. Such a condition may give the erroneous impression that the fertilization tube is formed by the egg. Interesting in this connection are the observations of King (16) on *Araiospora pulchra* Thaxter in which he described the fertilization tube as being of oögonial origin. Behrens (2), working on *Rhipidium europaeum* (Cornu) von Minden, a genus closely related to *Araiospora*, is inclined to think that King made a mistake on this point.

At this stage the egg nucleus has contracted greatly so that the nuclear material is contained mostly in a single, almost solid, deeply staining, spherical mass and on one end of this nucleus a narrow line of material extends out to a smaller spherical or lobed body, the centrosome, from which the astral rays radiate. The female nucleus of *Achlya* (Trow, 26, and Mücke, 20) and *Saprolegnia* (Claussen, 6) at this stage show similar peculiar structural characters to those just described for the female nucleus of *Leptolegnia*.

After the male nucleus comes in contact with the female, both increase greatly in size. The fusion of the two nuclei seems to be a rather slow process. Apparently they begin to fuse immediately after coming in contact, but fusion may not be completed until sometime later, perhaps two days. I have seen several eggs with a much thickened wall in which the fusion of the two nuclei was not yet complete. During this stage the supernumerary nuclei continue to degenerate until finally in the ripe egg no signs of them remain (figs. 23–27). The ripe egg is surrounded by an exceedingly thick wall and contains a single large nucleus (fig. 27).

After six weeks or two months some of the ripe eggs may be brought to germination, but I have been unable to stain the nuclei in the germinating eggs.

Comparison of the Development of the Sexual Organs of Leptolegnia with Other Related Fungi

The cytology of several species of *Saprolegnia* (Claussen, 6; Mäckel, 17), *Achlya* (Trow, 26; Mücke, 20; Patterson, 23), and one species of *Aphanomyces* (*A. laevis*) (Kasanowsky, 15) has been worked out and a striking uniformity in oögenesis has been found in all three of these genera except that in *A. laevis* Kasanowsky (15) describes a well developed coenocentrum comparable to the coenocentra found in the Peronosporaceae. In all of the species of *Saprolegnia* and *Achlya* which have been investigated, the

oögonia contain a number of eggs, but even where only one egg is formed, as is usually the case in *A. caroliniana* Coker, oögenesis is similar to that in those species in which there are several eggs.[3] In these genera the young oögonia contain a large central vacuole and a peripheral layer of cytoplasm in which are imbedded many nuclei. The nuclei undergo one mitotic division and then all degenerate except one for each egg origin. The cytoplasm now heaps up around each egg nucleus forming the egg origins. The peripheral membrane between these is thinned down until it breaks at one or more points and the egg origins then quickly recede from the wall to coalesce more or less in the center. If the egg origins are numerous and the oögonium comparatively small, the origins may be so closely pressed to each other as to disappear almost completely. They quickly form again, now taking on a spherical shape. In the formation of the egg origins all of the genera in which oögenesis has been investigated differ markedly from *Leptolegnia*, for in the latter this structure is in the center of the oögonium and the vacuoles are many and peripheral; whereas in the former, and this holds where only one egg origin is present, the origins are peripheral and the vacuole single and central. The migration of the supernumerary nuclei to the periphery and their degeneration in this position is also strikingly different from the course of events in all other members of the *Saprolegniaceae* so far investigated. Although there are no signs of a true periplasm in *Leptolegnia*, it is possible that the degenerating nuclei take part in the formation of the thick wall around the egg. Another noticeable point of difference between *Leptolegnia* and *Saprolegnia*, *Achlya*, and other genera, is in the absence of a fertilization tube in the former and the presence of such tubes in the latter genera.

The egg structure of *Pythiopsis cymosa* as shown by deBary (13) is somewhat like that of *Leptolegnia* (deBary does not show a completely ripe egg of *Leptolegnia*) in the possession by the former of an eccentric group of oil globules. The arrangement of the oil globules is quite similar in the eggs of the two genera, and it is probable that a careful study of oögenesis in *Pythiopsis* will show a striking relationship with *Leptolegnia*.

In the process of oögenesis, *Leptolegnia* appears to be closely related to certain of the Peronosporaceae. The thick-walled egg, always single in the oögonium, and the migration of the supernumerary nuclei to the periphery to degenerate are characters of importance which *Leptolegnia* has in common with this family. Some of Murphy's (22) figures for *Phytophthora erythroseptica* Pethy., notably figures 18, 20, and 24, are very much like similar stages in *Leptolegnia* (figs. 18, 24), and some of Miyake's (19), or Patterson's (24) figures for *Pythium* are also much like *Leptolegnia*. It is difficult to say which of these two genera *Leptolegnia* resembles most in sexual reproduction. It is obvious, however, that in sexual reproduction *Leptolegnia* is closer to *Pythium* and *Phytophthora* than to any of the Saprolegniaceae.

[3] In *A. caroliniana* Coker there are no antheridia and fertilization does not occur.

The process of oögenesis in the Leptomitaceae as represented by *Araiospora pulchra* (King, 16) and *Rhipidium europaeum* (Behrens, 2) indicates a close relationship to the Peronosporaceae, especially the Albuginaceae, whereas *Leptolegnia* is closer to *Phytophthora* and *Pythium*.

In trying to determine relationships, asexual reproduction must also be considered. In the possession of long, filamentous sporangia with zoöspores usually in a single row, *Leptolegnia* shows a resemblance to *Aphanomyces*, In view of the striking differences in sexual reproduction in the two genera, however, it is doubtful if this similarity of sporangial structure is of any phylogenetic significance. In the behavior of the zoöspores, *Leptolegnia* resembles *Saprolegnia* more closely than any other of the water fungi.

In view of the fact that in sexual reproduction *Leptolegnia* resembles *Phytophthora* and *Pythium* more closely than any of the Saprolegniaceae, but that in asexual reproduction and the coarseness of the vegetative mycelium it resembles certain of the Saprolegniaceae more closely, it appears that this genus is a sort of connecting form between these two families. It is highly probable that as the water and soil fungi are more widely studied, new forms will be discovered which will throw additional light on the rather obscure relationships of *Leptolegnia*.

Summary

Leptolegnia caudata deBary is one of the rare but widely distributed members of the Saprolegniaceae. The fungus is usually obtained only in the sterile condition and when sexual organs do occur they are quite spasmodic in their appearance, i.e., of a number of cultures made at the same time some may show sexual organs while others do not. When the sexual organs do appear spontaneously, they usually occur in groups or clusters. Just why some cultures may produce sexual fruits whereas others though treated apparently in the same way do not produce any, and why the organs are so often in clusters, remain unexplained. The mycelium is homothallic as is proved by single-spore cultures and by attempts to cross strains collected from various localities.

Sexual reproduction may be influenced by a number of factors. High temperature (27–35° C.) totally inhibits it. In certain strains low temperature, 14° C., may be influential in causing the formation of sexual organs. Light does not appear to have any influence on sexual reproduction. After considerable experimentation with nutrients, one solution was found which would produce sexual organs with certainty and in great numbers. The fungus was grown in this solution (0.5% maltose; 0.1% meat peptone) for two to three days, or until a vigorous mycelium was formed, and then transferred to sterile water in which after a few days the sexual organs appeared. Some of the results seemed to indicate that the H-ion concentration influenced sexual reproduction.

The antheridial and oögonial threads are apparently of diclinous origin.

The antheridial branches twine about the oögonial branches. One or several antheridia become applied to each oögonium. The young oögonium and antheridium are multinucleate, the former containing about 30–40 nuclei, the latter about 6. The nuclei of both organs undergo one mitotic division, this division usually occurring at night. In the oögonium all of the nuclei except one migrate to the peripheral region and there degenerate. The remaining nucleus takes up a central position, develops very distinct astral rays, and becomes the female gamete nucleus. At this stage the protoplasm surrounding the female gamete nucleus is homogeneous and dense, while in the peripheral region the protoplasm is more vacuolated and contains the supernumerary nuclei in various stages of disintegration. The antheridial contents also become differentiated into a dense, uninucleate, central plasm and a peripheral, vacuolated multinucleated plasm. The antheridial and oögonial walls become hydrolyzed and the enlarged central nucleus of the antheridium, along with some of the cytoplasm, passes slowly into the oösphere. No antheridial tube is formed but the male nucleus leaves a distinct path through the oösphere as it passes to the female nucleus. Fertilization appears to take place, as a rule, in the afternoon.

As the egg ripens the male and female nuclei fuse, and the wall becomes very thick. After a rest period of a month to six weeks the eggs may germinate. I have been unable, however, to stain the dividing nuclei in the ripe eggs.

Because of the closer similarity between oögenesis in *Leptolegnia* and certain of the Peronosporaceae (*Phytophthora* and *Pythium*) than between *Leptolegnia* and other members of the Saprolegniaceae it appears that *Leptolegnia* represents a connecting form between these two families. It seems best, however, to retain the genus in the Saprolegniaceae because of the similarity in vegetative structure and asexual reproduction.

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EXPLANATION OF PLATES

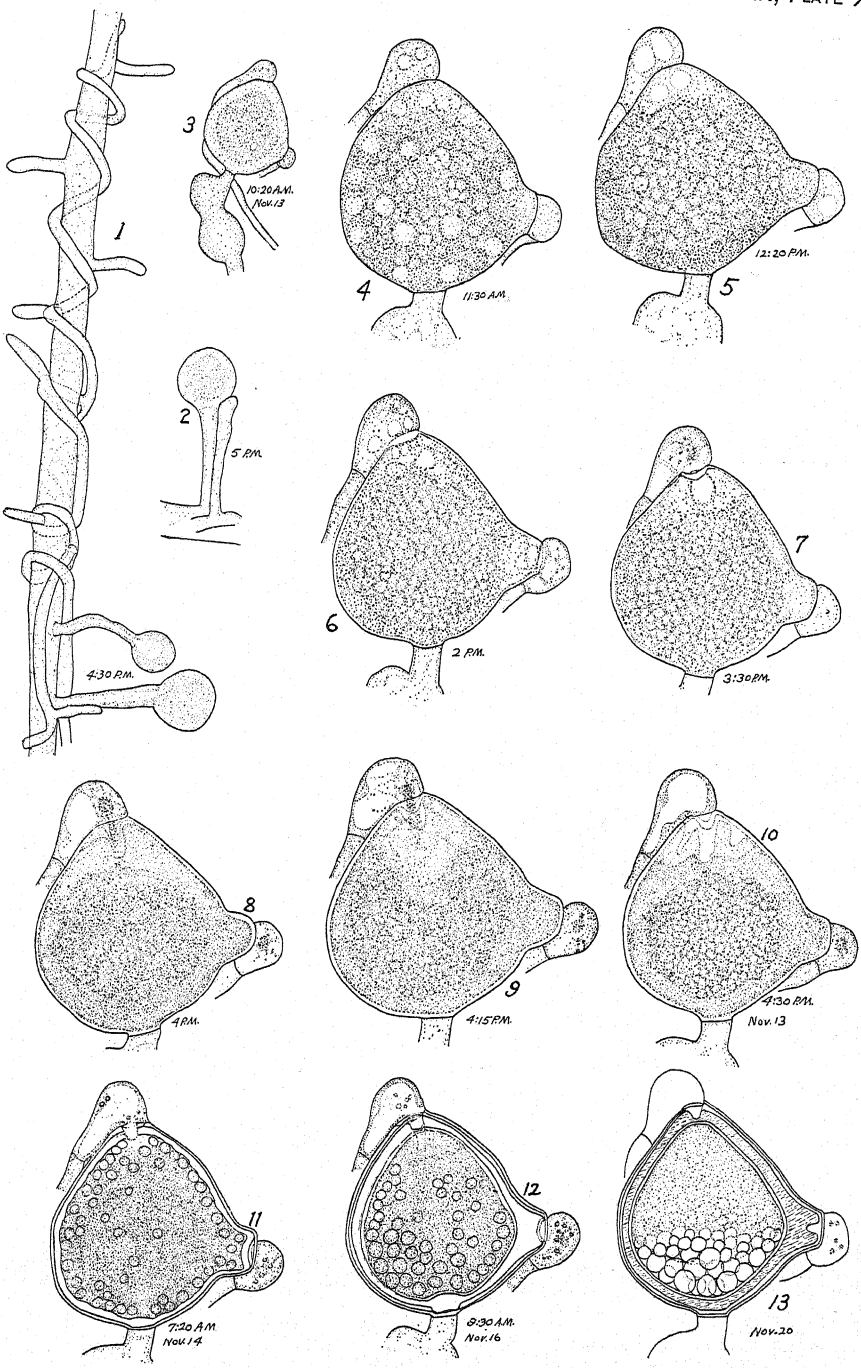
The figures on plates XLIII and XLIV, excepting figure 16, were drawn with a Zeiss 2 mm. apochromatic objective N. A. 1.40 and compensating ocular no. 18, at table level with the aid of an Abbé camera lucida giving a magnification of about 3500. On reproduction the drawings have been reduced considerably over one-half.

Plate XLII

Fig. 1. Oögonial branch with two young oögonia and antheridial branch twining about it. Note antheridial branch creeping out oögonial stalk. × 280.

Fig. 2. Same oögonium and antheridial branch as shown at the lower part of figure 1 half an hour later. × 280.

Figs. 3–13 show stages in the development of the same oögonium from 10:20 A.M., Nov. 13–Nov. 20. Fig. 3, × 280, others × 660.


1
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2
5 P.M.
3
10:20 A.M.
Nov. 13
4
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6
2 P.M.
7
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9
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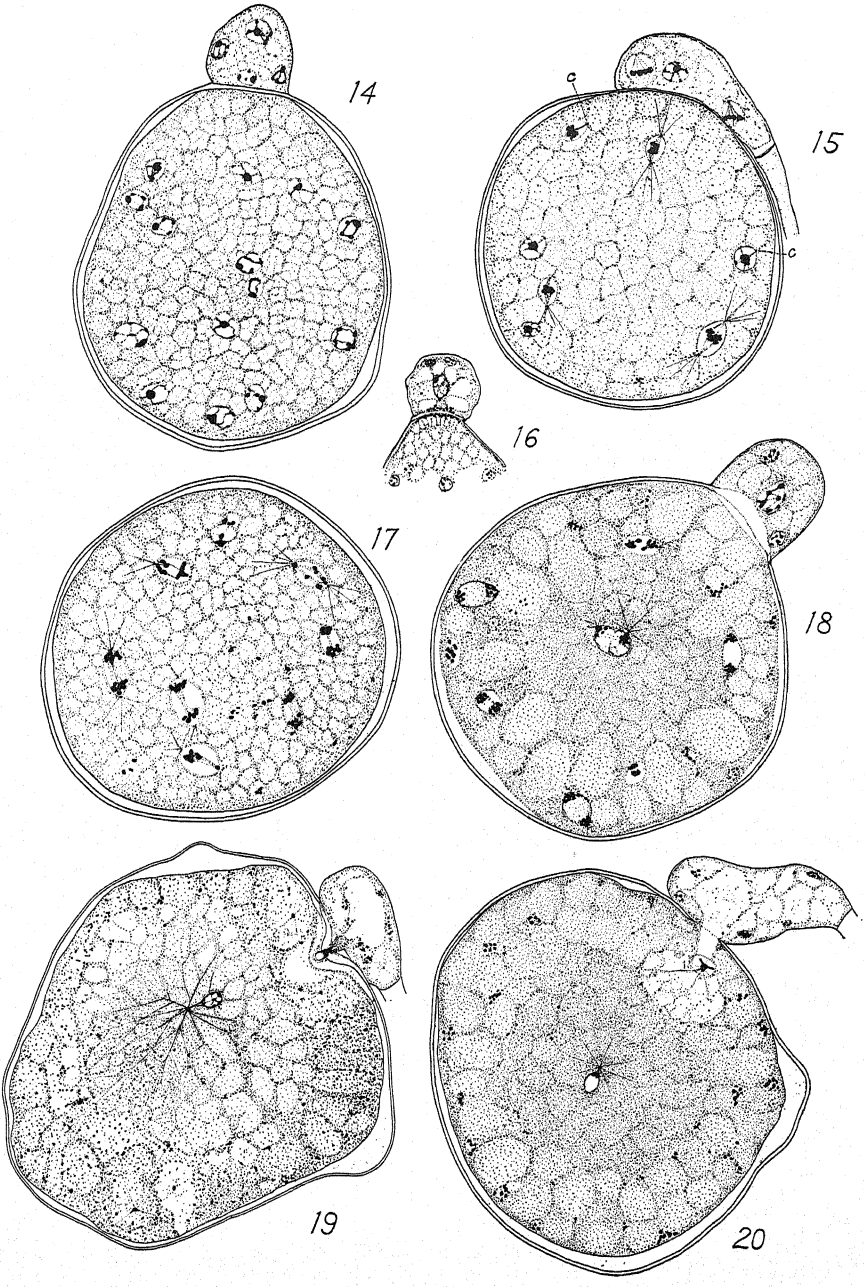
Nov. 16
13
Nov. 20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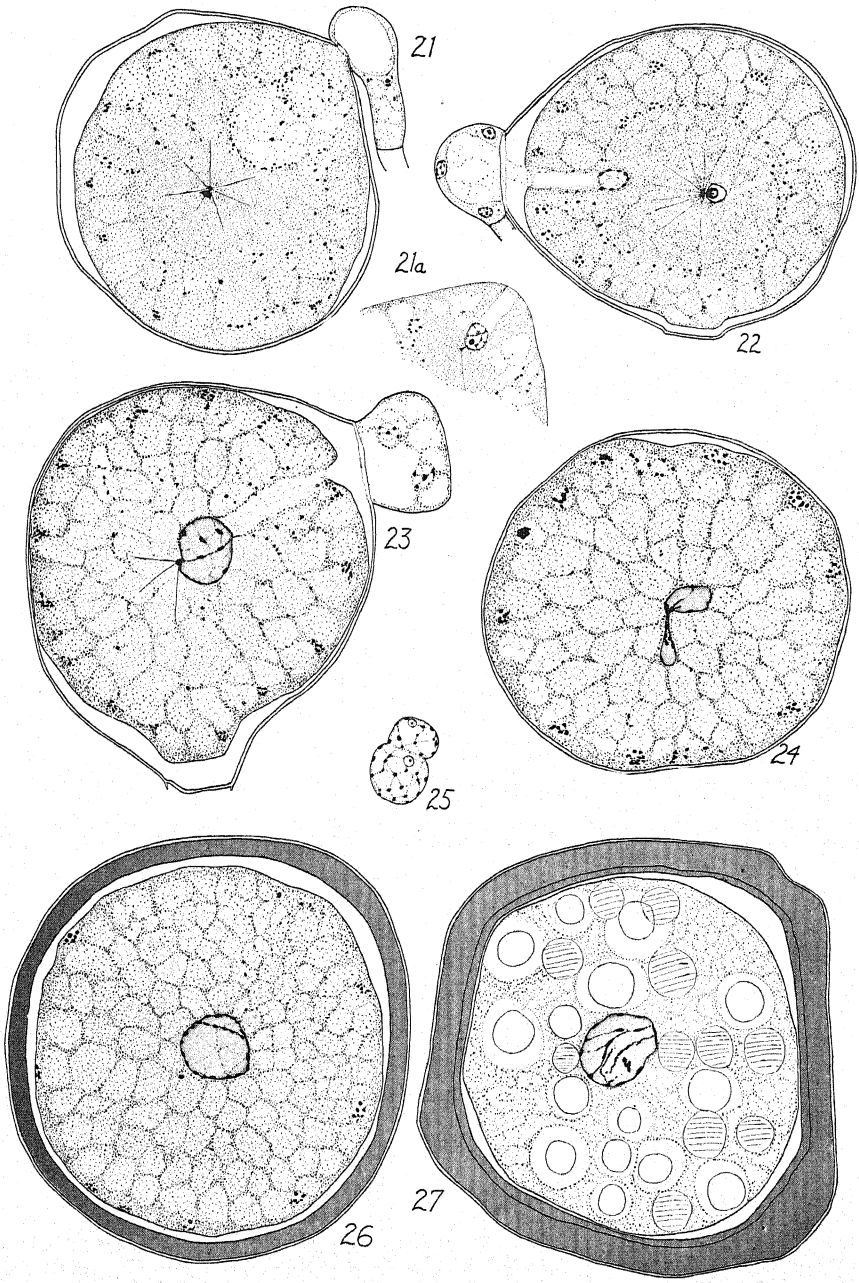

COUCH: LEPTOLEGNIA

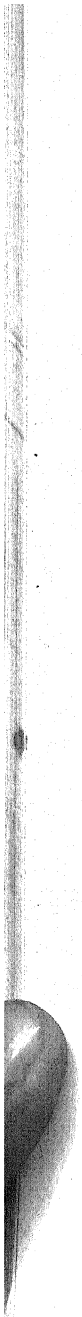

FIG. 3. Beak-like extensions forming on oögonium beneath antheridia.

FIG. 4. Oögonium and antheridium vacuolated.

FIGS. 5 AND 6. Hydrolysis of membrane between oögonium and antheridium.

FIG. 7. Beginning of passage of antheridial material into oögonium. Vacuolar material passing over. Note the central, denser area in the antheridium.

FIGS. 8 AND 9. Passage of gonoplasm into oögonium. Note the lighter region in oögonium beneath antheridium.

FIG. 10. Discharge of gonoplasm completed. Note peripheral layer of cytoplasm; antheridium on side failed to discharge.

FIGS. 11, 12 AND 13. Stages in ripening of egg. Note development of thick outer egg membrane and thin inner membrane. Oil globules flow together, collecting at one side of protoplasm.

PLATE XLIII

FIG. 16, × 720, the rest × 1400. FIGS. 16 and 19 from iron alum haematoxylin preparation, the rest Gram's gentian violet method.

FIG. 14. Young oögonium and antheridium. Most nuclei in resting condition, some in early stages of mitosis. Nuclei scattered throughout oögonium.

FIG. 15. Most nuclei in peripheral region, in metaphase, astral rays evident. *C*, centrosome.

FIG. 16. Early stage in hydrolysis of oögonial and antheridial membrane. Note deeply stained granules bordering membrane and vacuoles.

FIG. 17. Most nuclei in anaphase. Astral rays evident.

FIG. 18. Nuclei in telophase and all save one occupying a peripheral position. The central nucleus has about completed division and one of daughter nuclei is reorganizing as egg nucleus. Note straight astral rays. The partition between antheridium and oögonium seemed almost completely hydrolyzed. Male nucleus in center of antheridium.

FIG. 19. Iron alum haematoxylin preparation showing nucleus with elongated process from distal end of which radiate astral rays, extending straight at first but soon branching to form intricate network. Egg divided into two regions: central less granulated and peripheral region with many deeply staining granules. Vacuoles larger in peripheral region but extending throughout. Early stage in fertilization: antheridium discharging nucleus into egg through hydrolyzed membrane. No oögonial beak beneath this antheridium—a rather exceptional condition.

FIG. 20. Stage in fertilization slightly later than that shown in figure 19. Note straight astral rays and denser cytoplasm immediately around centrosphere. Note vacuole preceding male nucleus. No oögonial beak here, and hydrolyzed membrane not evident.

PLATE XLIV

FIG. 21. Slightly later stage than figure 20. Section through egg nucleus, apparently tangential. Note large vacuoles in egg beneath antheridium and empty space in antheridium.

FIG. 21*a*. Part of section of same oögonium as in figure 21 with male nucleus and path left by it in egg.

FIG. 22. Later stage in fertilization. Note characteristic oögonial beak beneath antheridium. Female nucleus in center with distinct astral rays. Male nucleus approaching egg nucleus leaving distinct path. Antheridium empty except for thick peripheral layer.

FIG. 23. Male and female nucleus fusing, both greatly enlarged, as characteristic at this stage. Path left by male nucleus still evident.

FIGS. 24, 25. Stages in fusion of male and female nucleus.

FIG. 26. Late stage in nuclear fusion and in thickening of outer layer of egg wall.

FIG. 27. Nearly mature egg, showing large fusion nucleus, globules of food material and thick two layered membrane all surrounded by oögonial wall. A completely ripe egg is not shown.

INTRACARPELLARY FRUITS AND OTHER CENTRAL PROLIFERATIONS OF THE FLORAL AXIS IN *HIBISCUS*

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Some interesting examples of central proliferation of the floral axis have been observed in hybrid forms of *Hibiscus*, grown commonly in Hawaii as ornamental plants. In most of the cases previously described either the carpels were absent or the proliferation had developed from between the carpels and extended beyond them. Masters (5) cites instances in which an intracarpellary flower, as a central proliferation of the axis, was produced. A number of cases have been described in which the proliferated floral axis produced a more or less completely formed secondary fruit which was entirely enclosed within the ovary of the primary flower. Harris (3) has described proliferations of this kind in *Passiflora* and cited an example of the occurrence of the same phenomenon in the fruit of *Carica papaya*. This abnormality is apparently of quite frequent occurrence in fruits of the papaya and of citrus (6). Delavaud (1) observed small capsules with aborted ovules within the ordinary capsules of *Hibiscus tiliaceus* L. Harris (4) has also reported the occurrence of intracarpellary fruits in capsules of *Hibiscus esculentus*. The included fruit appeared, from its position, to be a central proliferation of the floral axis but this point could not be determined with certainty.

Attention was first called to these intracarpellary proliferations in attempting to hybridize certain forms of *Hibiscus*. It was observed that the seed pods dropped off within a few days, as is frequently the case in crosses of *Hibiscus*. On opening the pods for examination as to seed development small, complete pistils were found in several of the pods. Externally these pods were strongly convoluted whereas ordinarily they are not. It was thought, at first, that the abnormal condition occurred only in flowers which had been pollinated. Subsequent observations, however, indicated that intracarpellary pistils may be found in flowers at the time of blossoming and that undeveloped proliferations are sometimes present in the buds. From this it appears that even self pollination is not necessary for the production of the intracarpellary proliferations.

The intracarpellary pistils observed were, in all cases, proliferations of the central axis of the capsules in which they were included. The proliferated central axis extended from the receptacle to about midway to the apex of the capsule. Both the proliferated axis and the included pistil at its summit were surrounded by the placenta. The size of the included

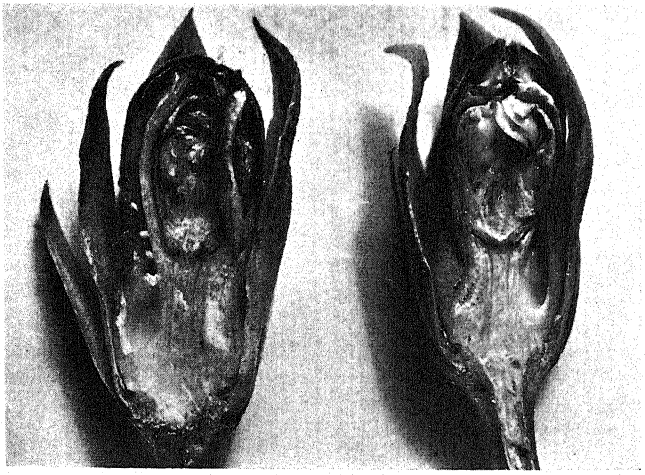
pistil varied with the age of the ovary within which it was included. Although pollination seems not to be necessary for the production of the intracarpellary proliferations, the size of the pistil produced within a capsule depends indirectly upon pollination. The pistil of flowers which had not been pollinated dropped off within two or three days after the corolla had fallen. When pollination occurred the pistils continued their development for a considerable length of time although none ever came to maturity.

TEXT FIG. 1. An intracarpellary pistil as a proliferation of the central axis in a pollinated pistil of *Hibiscus*. The ovules very rarely develop into seeds. × 5.

The included pistil, in the ovary of unpollinated flowers, was found to vary from 3 to 5 millimeters in length. The included pistils were green in color and had apparently normal ovaries but very short styles with no stigmas. In some cases, in which the flowers had been pollinated either by selfing or by artificial crossing, the intracarpellary pistils were larger than those just described, attaining a length of 8 to 10 millimeters. The ovary of these pistils was 3–4 millimeters long and 2.5–3 millimeters in diameter. The pistils were otherwise very similar to those first described but had always well developed styles and stigmas. The styles, which were 4–6 millimeters long, were united usually nearly to the stigmas but in some instances one or more styles were entirely separate. The stigmas were spherical, pubescent, and yellow as in normal stigmas of plants of this particular parentage. The styles and walls of the ovary were also pubescent with the same kind of hairs found on normal pistils. These larger intracarpellary pistils were always found in the upper part of the capsule (text fig. 1). With the enlargement of the included pistil it usually broke through

the placenta into one of the locules. The styles were crowded against the top of the ovary within which they were enclosed, which caused them to turn laterally and downward along the ovary wall. Ovules were present along the entire length of the placentae in all the specimens observed. The proliferations here described are of the same type as that described by Harris (4) in *Hibiscus esculentus*. Harris was unable to satisfy himself "of the existence of a stalk directly connecting the included fruit with the torus," the presence of which was very apparent in all specimens found by the writer.

A second type of proliferation, which sometimes occurs in *Hibiscus*, is that of the production of a second flower as an extension of the central axis of the primary flower. Proliferations of this type are of common occurrence in many kinds of flowers and especially in roses. Typical cases have been



TEXT FIG. 2. A secondary flower as a proliferation of the central axis. The secondary flower is entirely enclosed within the capsule at the time of opening of the primary flower. × 2.

cited by Masters (5) and by Worsdell (8). An apparently similar case is mentioned by Wilcox and Holt (7) in a red-flowered *Hibiscus* of the parentage Single Pink (Experiment Station) × Carnation. In the specimens observed by Wilcox and Holt the secondary flower, during the early stages of development, was wholly enclosed within the ovary of the primary flower. As development proceeded the enclosed flower broke through the ovary wall and projected well beyond it so that the ovary wall occupied the position of a calyx. Another example of this kind has been found by the writer in a yellow-flowered *Hibiscus* of unknown parentage. At the time of opening of the primary flower the secondary flower was completely enclosed within the ovary of the primary flower (text fig. 2). After the petals of the primary flower had fallen the secondary flower broke through the ovary wall and

emerged as a smaller very double flower. The presence of stamens or of a pistil in the secondary flower was not observed.

These different types of proliferation are always found on plants resulting from crosses of definite parents. In no instance have proliferations or other floral abnormalities been found on plants of any species or variety non-hybrid in character. Nor, in any instance, have two different types of proliferation been found on the same plant or on plants of the same parentage. On the other hand, plants resulting from crosses of very different parents may produce essentially the same type of proliferation. As an example of this may be mentioned the flowers described by Wilcox and Holt (7) and those found by the writer. The parents in the two cases were undoubtedly different. The parentage of the flowers described in this paper is unknown but at least one of them was yellow flowered. In those cases in which plants resulting from crosses of different parents have produced proliferations similar in their general features, where direct comparison could be made, minor differences have always been found indicating the difference in parentage.

The cause of these abnormalities seems to be a disturbance brought about by hybridization. The disturbance is probably due to inharmonious elements or factors in the gametes derived from plants of different species, as was found by Goodspeed and Clausen (2) in certain *Nicotiana* hybrids. Many different species of *Hibiscus* have been crossed to produce the cultivated forms grown in Hawaii. Among them some species display such a high degree of mutual incompatibility that they cannot be crossed. In other species, between which crosses can be made, the gametes contain a sufficiently great proportion of inharmonious elements to cause sterility, varying from partial to complete, and to cause disturbances which presumably result in the various floral abnormalities observed.

Summary

Small, perfectly formed pistils and "double" flowers as intra-ovarial inclusions in capsules of *Hibiscus* are described.

Amherst,
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POLLEN-TUBE GROWTH IN PRIMARY AND SECONDARY $2n + 1$ DATURAS

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The writers have been collaborating since 1921 in a study of pollen-tube growth of the various $(2n + 1)$ mutants of *Datura*. One objective was the use of this material as a means of discovering experimentally some of the methods of elimination of various kinds of 13-chromosome gametophytes in the process of gametophytic selection (9) and of evaluating this process as a mechanism of evolution. A more practical purpose was the desire to discover the special conditions, if any, under which $(2n + 1)$ types may be transmitted through the pollen and whether the differential elimination of male gametophytes is subject to experimental control.

A study of several other phases of developmental selection—interovular selection as well as gametophytic selection—had been begun (11) when a special technique was developed by which pollen-tube growth could be investigated. Our interest and effort was, therefore, directed very largely to the study of male gametophytic selection, especially since it was found that this technique was adapted to a quantitative study of pollen-tube populations, and since it was useful for the study of the effects of certain genes (12) as well as of extra chromosomes on pollen-tube growth.

The investigations included in this paper are based on a series of counts of the ungerminated pollen grains and on the distributions of the ends of normal and abnormal pollen tubes in individual styles after pollinations with the pollen taken from various 25-chromosome plants.

The styles used in all of these comparisons were those of normal $2n$ plants from Line 1 *A*. Normal $2n$ styles were used because we believed that the results would not be comparable if the styles were different, as would be the case if the results were taken from selfing the various $(2n + 1)$ plants. The mutants tested were all in Line 1 *A*. To have selfed each mutant in addition would not only have greatly increased the labor, but would also have complicated the experiment further because the styles of many of the $(2n + 1)$ mutants differ in structure and degree of maturity, and some are very difficult to dissect. When tests of the styles of selfed flowers were made in our preliminary studies, the results appeared to be the same as those from the pollinations on $2n$ styles.

A series of tests was made in 1923 using normal pollen from $2n$ plants on the styles of each of the various $(2n + 1)$ mutants, but no definite evidence was found which would indicate any significant differences in pollen-

tube growth when such different types of styles were used. Moreover our tests of the pollen from $2n$ plants on the styles of $4n$, $3n$, and n plants showed us later that the pollen of $2n$ plants grows well on the pistils of all of the different balanced types (16).

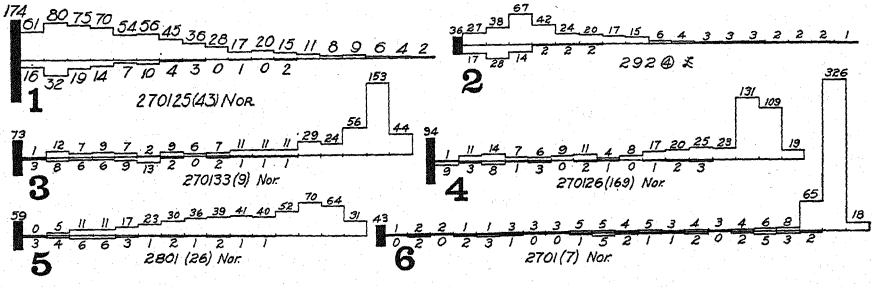
All of our experience has convinced us that the best and most reliable comparison of the growth of pollen tubes of $(2n + 1)$ types could be made by the pollination of $2n$ styles with the pollen of the mutant types, and the results presented here were all obtained from tests made in this manner.

The graphs or diagrams of our figures were made by counting the ends of the pollen tubes and plotting them in their appropriate 2-mm. interval of distance from the stigma at the left, representing the pollen-tube population growing from the left toward the right. Ungerminated and burst pollen grains observed on the stigma were plotted in the vertical bar at the left, with normal-appearing pollen tubes above the datum line and abnormal-appearing, swollen, or burst pollen tubes below this line.

Pollen-tube Growth in Normal Plants

We have described pollen-tube growth in normal plants in several publications (10, 12–17). There are, however, a number of details of procedure which should be given here. We have frequently made the qualifying statement that the tests concerned in our comparisons were obtained under "favorable conditions" without explaining fully what may constitute unfavorable conditions for the tests.

Figure 1 shows a test of pollen-tube growth in a $2n$ plant under conditions which we have attempted to avoid whenever possible. Figure 2 shows a similar test from a selfed Line 2 greenhouse plant. We have observed this type of distribution in various types of normal plants when obtained under



Text Figs. 1–6. Explanation in text.

non-setting conditions. The early flowers of plants growing in the garden in rich soil sometimes give this type of pollen-tube distribution, and in greenhouse plants growing vigorously this condition may be found in the first flowers, though in potted plants the time and conditions of repotting, etc., may cause considerable variation in this respect. At times early in the season, a much larger proportion of the pollen tubes are found burst

within the style. This non-setting condition may also be indicated by a poor germination of the pollen. It seems that both the pollen used and the conducting tissue in the pistils of unfavorable plants may contribute to this peculiar behavior. One is not likely to obtain many tests in which the stylar tissue is the factor, since the styles of such plants are usually difficult to dissect.

There is greater danger in obtaining this abnormal distribution when the pollen is maturing on plants not in the best condition. Our criterion in judging the best time to test the pollen and to avoid the undesirable conditions resulting in text figures 1 and 2 was the abundant setting of seed capsules on the pollen parent. At times when normal plants tend to give pollen-tube distribution curves which are not typical, many of the buds, flowers, or young seed capsules drop off the plants.

Though a high percentage of ungerminated pollen may be found in test pollinations from plants growing under non-setting conditions, this fact does not always indicate that the ungerminated pollen is incapable of germination. We take it that distribution curves such as those of figures 1 and 2 indicate that there was an uneven germination of the pollen; that the period of germination was spread over a long time-interval. The stigma, though fully grown, was probably not fully matured, and much of the pollen was more or less delayed in germination. Some of the pollen grains may have been partially immature, though they were shedding or nearly ready to be shed from the anthers when used. It seems reasonable to conclude from these facts that such results are due to the physiological condition of the plant, and that both the pollen and the pistils may be affected by these internal physiological conditions. Of course, not all failure in fruit-setting in plants should be attributed to failure of adequate pollen-tube growth, since we have found many seed-capsules fall off in crosses in which we had definitely been able to determine the fact that an abundance of pollen tubes had reached the ovary. However, we do feel that inadequate pollen-tube growth may at times be the principal cause of the difficulty in the setting of fruit (13), and we are convinced that investigations on this phase of the problem of fruit setting in economic plants would be profitable.

In figures 3 to 6 and in figure 20, we are giving some representative examples of distributions of pollen-tubes in normal plants under conditions favorable for testing. In some of these, the number of pollen grains applied to the stigma was much greater than in others but in no case were there any serious competitional effects due to crowding.

It will be noted that while figures 1 and 2, which represent unfavorable conditions for testing, show a backward skew in the curve of distribution, all the other figures have a pronounced forward skew, figure 6 representing the extreme in this respect. Figure 5 has a less pronounced forward skew and shows a considerable number of short tubes.

These tests (figs. 3–6) were selected from among a series of control

pollinations made at various times during the testing of the pollens from ($2n + 1$) types, and indicate that on the whole the styles used in making tests of the chromosomal types were giving favorable pollen-tube growth conditions when tested with n pollen.

Usually the tests which we made with the pollen of 25-chromosome plants, themselves, show whether the conditions of pollen maturity were near optimum. There is always a large group of n pollen grains which give pollen tubes grouped in a distinct mode, if the conditions of testing are satisfactory. Thus each test of the pollen of a ($2n + 1$) plant has a control embodied within it.

Experimental Methods and Precautions

As our experience in the study of pollen-tube growth increased, we found it necessary or desirable to institute certain experimental precautions in our routine work. These may be worth the consideration of anyone attempting to carry on similar experiments, and there are doubtless other refinements which should be made in the procedure.

Test Flowers. The flowers of $2n$ plants used for making the tests of pollen-tube growth were carefully selected from healthy plants which were at the time setting seed capsules. Small flowers or flowers which had abnormally developed pistils were avoided. The flowers were castrated in the garden during the morning just before the pollen would have shed, or on the preceding evening, and their stigmas protected from desiccation and from visiting insects. As a protection we have employed straws, sealed at one end, inserted between the corolla and the style and tied to the corolla with a cord. The test flowers were left in the field until late afternoon (usually about 5 p.m.), the time when their corollas would have unfolded had they been left undisturbed. They were cut from the plants with a wet knife, their peduncles stood in small shell vials in water and brought into the laboratory, to be pollinated on the same evening. Freshly cut flowers, collected as outlined above, have given entirely satisfactory pollen-tube growth curves. These cut flowers differ from those left attached to the plant only in giving distribution curves with fewer burst pollen tubes and in giving a better pollen germination. The chief advantage from the use of cut flowers comes from the ability to control the distance of pollen-tube growth by using a nearly constant temperature (14) and by selecting a suitable time interval. The humidity may also be controlled, a very important factor which ensures prompt pollen germination. The tests were usually made for 12-, 15-, or 18-hour periods.

Selection of Pollen. As stated above, it was considered very important to collect the pollen to be tested from the plants that were at the time setting seed-capsules. Flowers which dropped off when touched, or which could be easily pulled off, were usually discarded and not used as a source for pollen, except when it was desired to test specifically the effect of this condition. The pollen-flowers with unopened anthers were gathered during the day, usually before noon, and stored with their pollen undisturbed until the time of pollination.

Pollination of the Test Flowers. Pollinations were usually made about 9 p.m. with small sable brushes. Different sizes were selected: No. 3 sable for abundant pollinations, Nos. 1 or 2 for more sparse pollinations. It is necessary to use a separate brush for each pollination, since sterilization with alcohol may render them toxic to the pollen for many hours afterward. This precaution of complete sterilization must, of course, be taken in making pollinations on garden or greenhouse plants from which seeds are desired. However, for tests of pollen-tube growth on styles, we have found it possible to free the brush of prac-

tically all pollen by alternately striking it sharply against a stone several times and rubbing it against a clean cloth. It is very improbable that a few scattered pollen grains which might be left on the brush would be included in the subsequent pollination and even if there were a few foreign pollen grains they could not perceptibly affect our results. However, when the pollen of triploid plants is used after making a series of pollinations with normal pollen, there is a possibility that the contamination of even a few foreign grains from the normal pollen previously used might affect the results (16). In tests of this kind, therefore, we have always employed brushes previously sterilized and thoroughly dried out.

There is, moreover, danger from pollen floating in the air even though *Datura* pollen is fairly heavy and adhesive. The unpollinated test flowers, both before and after pollination, should be adequately protected from floating grains and the pollinations should be made where there are no air currents. We have used the subterranean artificial cave in the laboratory of the Carnegie Institution at Cold Spring Harbor for this work. The advantages which it affords are nearly constant temperature (17.5° to 19° C.), freedom from air currents, and high relative humidity (13, 14).

The pollen was very evenly distributed over the stigma and an effort was made to avoid clumps of pollen. The stigmas of the test flowers were matured and receptive, which, together with a high humidity, usually gave almost immediate germination of the pollen grains which were capable of germination. These are very important conditions since we were not only testing the germination of the pollen composed of two or of several classes of pollen grains, but we were conducting a gametophytic race, as it were, in which the distance of growth in a given period was a measure of the differences between the various components of the pollen. In any race or contest there should be an even start. Had we merely observed the conditions found in flowers which were naturally self-pollinated in the garden there might have been no distinct grouping of the pollen tubes because there would have been a long-drawn-out period of pollen germination and perhaps less favorable conditions of humidity, etc., or the grouping of pollen tubes might have been due to disturbances which would give the same result as several distinct and separate pollinations of the stigma. Our test flowers were left entirely undisturbed between the time of pollination and killing. A bi-modal grouping of the pollen tubes in a style such as the one shown in figures 40, 42, and 44 may be obtained artificially with pollen of $2n$ plants by pollinating the stigma twice with an interval of 6–8 hours between the pollinations (18).

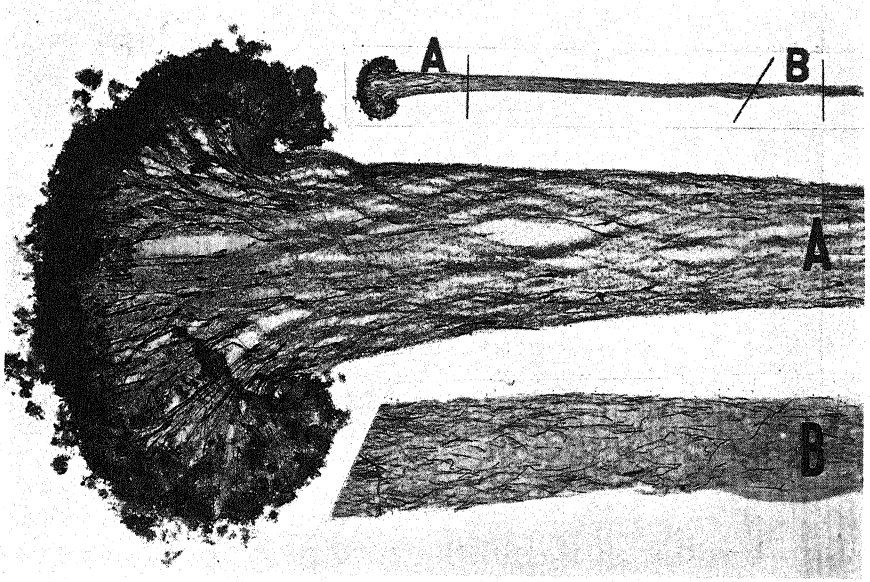
The procedure in slitting and killing the styles, dissecting, staining and mounting them has been described elsewhere (10, 13) and will not be repeated here, since there have been no very important improvements in these general methods.

In the various tests which we are describing we are using a slightly different set of symbols from those given in previous publications on pollen-tube growth (15, 17). In this scheme (4, 5, 8, 19) the ends of each chromosome have been numbered beginning with the largest or Rolled chromosome (4) which receives the numbers 1·2. The next ones are 3·4, 5·6, etc., up to 23·24 for the smallest chromosome. Our readers should thus bear in mind that what was formerly described (15) as the primary ($2n$ + Rl) may also be designated $2n + 1\cdot2$; its secondary Polycarpic ($2n$ + Py) may be written to show its doubled half as $2n + 1\cdot1$ and its complementary secondary Sugarloaf ($2n$ + Sg), may be written $2n + 2\cdot2$, while a ($2n$ + 1) plant has reference to any 25-chromosome plant. The size classes of the chromosomes of *Datura* have been described (1, 4).

The Rolled Group

Rolled (Rl) is the primary type in which the extra chromosome belongs to the largest size class, L.

The photomicrograph of text figure 7 shows a test preparation in the stigma region in *A*, and also the region of the forward group of pollen tubes in *B*. This is typical for the condition as shown in figures 8 to 10. The two classes of pollen grains with formulas n and $n + 1 \cdot 2$ are therefore definitely separated into two groups of gametophytes, differing in their behavior. Figures 8–10 are mid-season tests made at the most favorable time. The



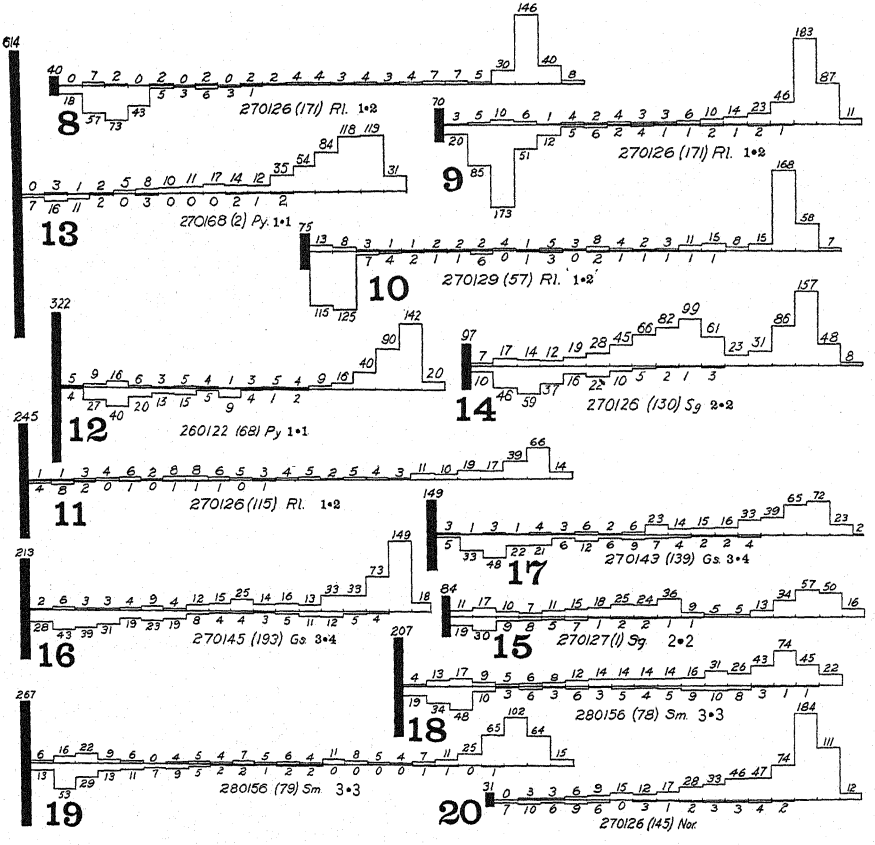
TEXT FIG. 7. Photomicrograph of a portion of a test slide of Rl. The ends of swollen and burst $n + 1$ pollen tubes are shown in A, near the stigma at the left, and the n pollen tubes at B. × 9.

part of the pollen with n chromosomes grows normally constituting the distinct forward mode, while the pollen with formula $n + 1 \cdot 2$ forms a group of abnormal-appearing pollen tubes, chiefly swollen or burst tubes remaining short near the stigma.

If the pollen of Rolled is tested early in the season (using pollen of flowers which would probably not have set seed capsules if left) the pollen-tube distributions frequently give the appearance of figure 11 Here the $n + 1 \cdot 2$ pollen failed to germinate, but the two groups of gametophytes of nearly equal proportions are nevertheless recognizable. Depending upon the condition of the plant whose pollen is tested, there may be slight variations in the distance through which the $n + 1 \cdot 2$ pollen tubes grow before they burst. This distance is recorded from the point at which the bursting takes place to the periphery of the stigma. In a series of many tests made every summer over a period of 10 years, however, this variation has fluctuated within only narrow limits.

At best the $n + 1 \cdot 2$ pollen tubes are able to grow only about 1/20 of the distance necessary to accomplish fertilization. Our tests indicate very definitely why the Rl type has never been successfully transmitted through the pollen.

The pollen of the secondary Polycarpic ($2n + 1 \cdot 1$) gives the distribution shown in figure 12. As explained in greater detail elsewhere (15) the dis-



TEXT FIGS. 8–20. Explanation in text.

junction of the trisome gives four classes of microspores having formulas, n, $n + 1 \cdot 1$, $n + 1 \cdot 2$, and $n - 1 \cdot 2 + 1 \cdot 1$. The last-named class of microspores is deficient in the segment $\cdot 2$ and aborts before the pollen reaches maturity in the anthers, constituting "bad pollen" (6, 7). The other three classes are present in the matured pollen and may be recognized in the distribution diagrams of figure 12. The n pollen has formed the pollen tubes of the forward mode. The $n + 1 \cdot 2$ pollen is recognized by its behavior in that it bursts in the style near the stigma, as this pollen class was found to do in the tests of Rolled, and is represented as abnormal pollen

tubes below the datum line at the left near the stigma. The $n + 1 \cdot 1$ group of gametophytes is recognizable as the ungerminated pollen. Neither of the last named groups complete the growth necessary for the pollen transmission of the extra chromosome which they carry. Therefore, the Polycarpic chromosome $1 \cdot 1$ cannot be transmitted as an extra through the pollen.

Differences are also found in the tests of the pollen of Polycarpic, depending upon the growth conditions of this 25-chromosome type. A test of pollen-tube growth from a field plant is shown in figure 13. Figure 12 is from a greenhouse plant growing under more favorable conditions when seed capsules were being formed.

The pollen of the secondary Sugarloaf ($2n + 2 \cdot 2$) gives the tests shown in figures 14 and 15. The disjunction of its trisome gives microspores of formulas n, $n + 2 \cdot 2$, $n + 1 \cdot 2$, and $n - 1 \cdot 2 + 2 \cdot 2$. The latter are deficient and therefore abort as "bad pollen" (6, 7). The three classes n, $n + 2 \cdot 2$ and $n + 1 \cdot 2$ are all recognizable in the distribution diagrams, in which under favorable conditions only a negligible portion of the pollen fails to germinate. Under very unfavorable conditions a considerable portion of the pollen fails to germinate, presumably the $n + 1 \cdot 2$ pollen.

It is obvious that $n + 2 \cdot 2$ gametophytes may grow normally, but at a slower rate than the n gametophytes, about 2/3 as fast. The tests, therefore, give bi-modal distribution curves of normal-appearing pollen tubes in which the $n + 1 \cdot 2$ pollen tubes may also be recognized by their behavior in bursting. We have successfully transmitted the extra $2 \cdot 2$ chromosome through the pollen (15) using some of the methods described for the pollen transmission of Cocklebur (17). These three conditions of pollen-tube growth in the Rolled group, wherein the primary stands between the extremes represented by the two secondaries, have been previously described (15).

Glossy and Its Secondary

Glossy (Gs) is a 25-chromosome primary whose extra chromosome belongs to the size class 1. This chromosome is designated as $3 \cdot 4$. Only one of the secondaries has been obtained thus far, Smooth (Sm) having $3 \cdot 3$ as the extra chromosome.

Since Glossy is definitely known to be a primary it has only 2 classes of pollen grains, n and $n + 3 \cdot 4$. Pollen-tube distribution tests under favorable conditions are shown in figures 16 and 17. The normal-appearing pollen tubes, coming from the n pollen, constitute the forward mode. If we assume an equality between the number of n and $n + 3 \cdot 4$ pollen grains it is obvious that the rest of the gametophyte population is not all found in the same condition. There are not enough ungerminated pollen grains to offset all of the germinated pollen, likewise there are not enough abnormal pollen tubes to offset the normal-appearing pollen tubes. Furthermore, there is a small second mode of normal-appearing pollen tubes at about the

mid-point in figures 17 and 18, but this group is not found in all of our tests.

The only explanation which fits these facts seems to be that the $n+3\cdot4$ pollen germinates poorly, which accounts for the large proportion of ungerminated pollen grains; that when this class of pollen germinates the great majority of the pollen tubes burst, but that a few of the $n + 3\cdot4$ pollen tubes may still be found growing at about half of the rate of n tubes, after the period of these tests. Hence, if the $n + 3\cdot4$ pollen tubes all burst in an early stage of growth the small group at the mid-point would not be evident. This condition is, in fact, somewhat similar to the one found in the tests of the pollen of tricarpel (12) in which a gene is responsible for a bursting of a large proportion but not all of the pollen tubes which carry it.

The question of the pollen transmissibility of the $3\cdot4$ chromosome as an extra presents many problems. It appears that under the most favorable conditions a very small group of $n + 3\cdot4$ pollen tubes might reach the ovary provided the group of n pollen tubes which precedes it is not large enough to fertilize all of the eggs. The backward group would contain fewer than the number of $n + 3\cdot4$ pollen tubes indicated in the tests of figures 16 and 17, since there would doubtless be other losses to this group through bursting before the ovary is reached. Any $n + 3\cdot4$ pollen tubes which reach the ovary would fertilize eggs in the lower portion, and the resulting $2n + 3\cdot4$ zygotes would be found among the seeds situated in the lower portion of the capsule (17).

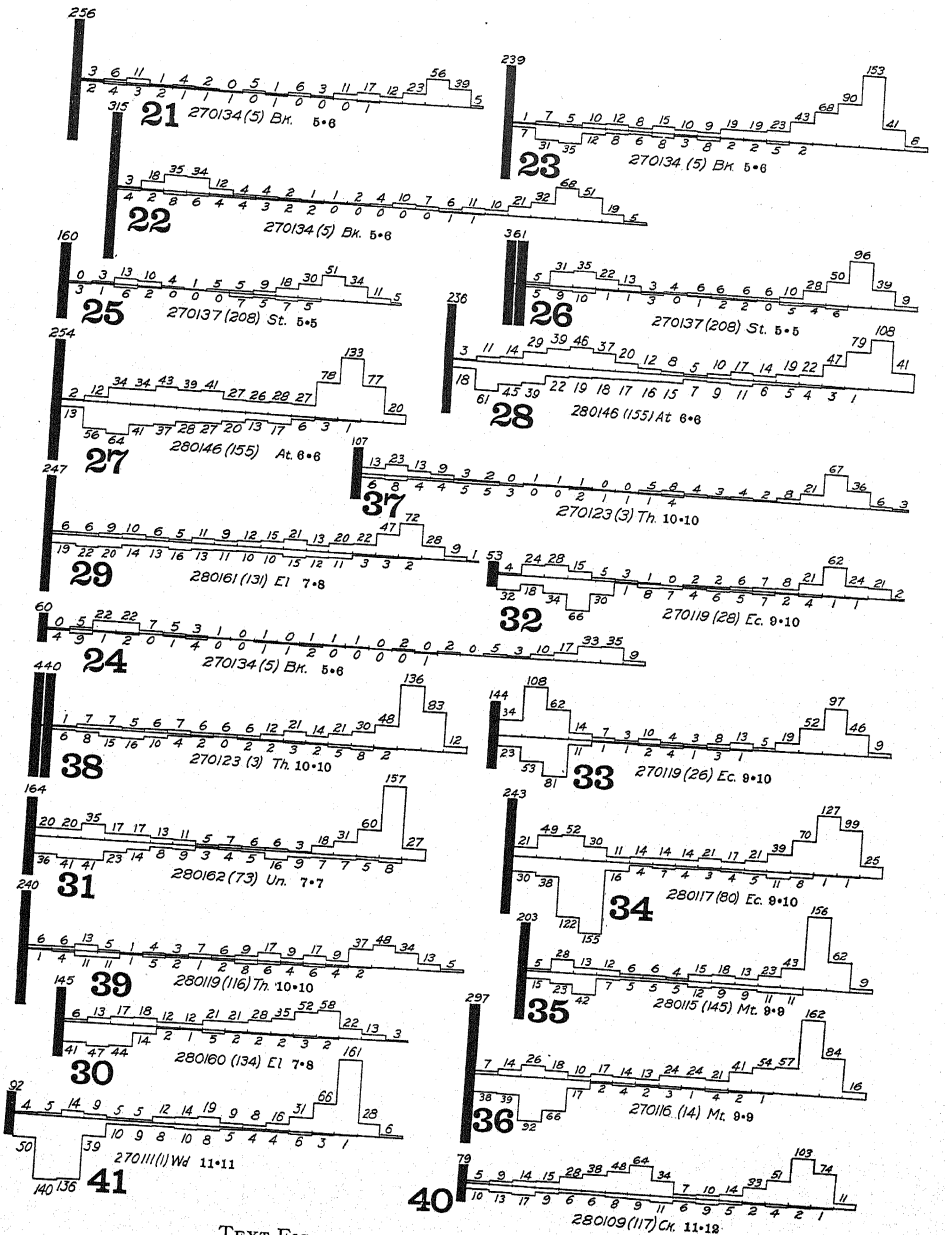
Tests of the secondary Smooth ($2n + 3\cdot3$) are shown in figures 18 and 19. Here a condition is found very similar to that of the primary, except that the abnormal pollen tubes tend to burst slightly nearer the stigma, and probably a smaller proportion of the $n + 3.3$ pollen grains actually germinate. Among the burst pollen tubes there are also those of the $n + 3\cdot4$ class which may be assumed to be distributed so that some remain growing in the small second mode seen near the mid-point as shown in figure 19.

The $3\cdot3$ chromosome, therefore, cannot be transmitted as an extra through the pollen.

The Buckling Group

Buckling (Bk) and its secondaries, Strawberry (St) and Areolate (At) constitute the second group (4) in which the extra chromosome belongs to the size class l. The Bk chromosome is represented with its ends numbered $5\cdot6$, that of St, $5\cdot5$, and At $6\cdot6$.

Figures 21 to 24 show several tests of Buckling. In a majority of the tests made through the growing season, half or nearly half of the pollen grains remain ungerminated on the stigma, as in figure 21. Actually most of the pollen grains are somewhat swollen and irregular, and often burst. We have plotted these as ungerminated pollen in our diagrams. Under the most favorable conditions which we have found, some very short pollen tubes, not burst, may be observed near the stigma (figs. 22 and 24), but



TEXT FIGS. 21–41. Explanation in text.

always associated with considerable ungerminated pollen. For some reason the 5·6 chromosome interferes with pollen germination, but if germination takes place the $n + 5{\cdot}6$ pollen tubes grow for only a few millimeters distance in the style. In tests made for 24 and 36 hours these short tubes are still near the stigma very abnormally swollen or burst, and that they sometimes burst within 12 hours is shown in figure 23. The Buckling chromosome 5·6 is, therefore, not pollen-transmissible as an extra under any known circumstances.

Strawberry (St) which has as its extra the doubled half 5·5 of the 5·6 chromosome is likewise not pollen-transmissible. Figures 25 and 26 show tests employing the pollen of St. As in the case of any secondary there are microspores of four classes forming pollen grains with n chromosomes, $n + 5{\cdot}5$, $n + 5{\cdot}6$, and $n + 5{\cdot}5 - 5{\cdot}6$. The last-named class being deficient, forms aborted pollen grains or "bad pollen." In some of the tests the short pollen tubes $n + 5{\cdot}6$ are recognizable, but the pollen $n + 5{\cdot}5$ apparently never germinates. The pollen of formula n, therefore, may be considered the only part in the pollen of St which becomes functional in fertilization.

Tests of Areolate (At), the other secondary of Buckling, with the doubled half 6·6 as the extra chromosome, presents a far more interesting set of distribution curves, as shown in figures 27 and 28. Here the proportion of ungerminated pollen is still considerable but there are two major modes of normal-appearing pollen tubes, the n group far in the lead, the $n + 6{\cdot}6$ pollen tubes in a retarded or slow-growing group. The $n + 5{\cdot}6$ pollen tubes from this secondary would be expected to be very short or to be represented by ungerminated pollen grains.

Areolate, therefore, is probably pollen-transmissible, but the $n + 6{\cdot}6$ pollen tubes grow only about a third as fast as the n pollen tubes. If the latter are not too numerous so that not all of the eggs are fertilized by them, we may expect to obtain some zygotes from the $n + 6{\cdot}6$ pollen tubes since the interval before the style drops off in a flower is usually several times as long as is necessary for the growth of n pollen tubes through the style. The lower portion of the seed capsule would contain the $2n + 6{\cdot}6$ plants. Areolate has only recently been recognized and the genetic back-crosses to test its pollen-transmissibility have not yet been planted.

We find, therefore, a striking similarity in the relations between the members in the Buckling group and in the Rolled group in which the primary stands between the extremes represented by the two secondaries.

Elongate and Its Secondary

The extra chromosome in Elongate (El) belongs to the size class M. Its ends are designated 7·8. The primary seldom sets seeds when grown in the open, and our most satisfactory tests of pollen-tube growth were, therefore, made by using the pollen of greenhouse plants. Only one second-

ary is known, Undulate (Un), which has the double half chromosome 7·7. The viability of Elongate is low as shown by the fact that only about 10% of its progeny (through female inheritance) are Elongates against more than twice this proportion in most of the other 25-chromosome plants (3).

Figures 29 and 30 show tests of the growth of the pollen-tubes of Elongate. These tests were made on the pollen of greenhouse plants. When the pollen of an occasional flower of a field-grown El was tested, about half of the pollen remained ungerminated. When we examine the distributions of pollen tubes in figures 29 and 30, we find that here again it seems necessary to consider ungerminated pollen grains together with the burst pollen tubes in order to account for enough $n + 7 \cdot 8$ gametophytes to offset the n pollen tubes. There is, moreover, a small second group of normal-appearing pollen tubes which are growing at about half the rate of the n group. If El only had better viability there might be a possibility under the most favorable conditions of a very limited pollen-transmission of the extra chromosome 7·8. The distributions of pollen-tube growth are, in fact, very similar to those which we have described for Glossy, except that Glossy has much greater viability (3), slightly above 23%, and the prospects of success in transmitting the chromosome 3·4 are, therefore, more than twice as favorable as they are for the 7·8 chromosome.

A test of Undulate (Un), the secondary of El, which has the 7·7 chromosome extra is shown in figure 31. It appears that a considerable portion of the $n + 7 \cdot 7$ pollen may germinate, giving rise chiefly to burst pollen tubes, and a group of very short normal-appearing tubes. Since this test was made from a field-grown plant, it is likely that the $n + 7 \cdot 8$ pollen grains which are to be expected in the pollen of this secondary are among the ungerminated pollen grains. In any event, the n pollen tubes are probably the only ones which may be expected to reach the ovary to accomplish fertilization. The short normal-appearing pollen tubes situated near the stigma seem to have burst in considerable proportions and still have a long distance to grow before reaching the ovary. Thus, we would not expect to obtain pollen-transmission of either Un or El.

The Echinus Group

Echinus (Ec) and its secondaries, Mutilated (Mt) and Thistle (Th), constitute a group involving chromosomes of the size class M. The Ec chromosome is represented with its ends numbered 9·10, that of Mt 9·9 and of Th 10·10.

Figures 33 to 36 show pollen-tube growth tests of Ec where various amounts of pollen are used. We always find a prominent group of burst pollen tubes in the funnel shaped portion of the style just below the stigma and a group of n pollen tubes far in the lead. Usually we find many normal-appearing pollen tubes which remain short among those which have burst.

Tests of Mutilated ($2n + 9 \cdot 9$) are shown in figures 35 and 36. Here we

consider the $n + 9 \cdot 9$ pollen grains the ones which fail to germinate. There are many burst pollen tubes of the type found in Ec in all Mt tests, but these would be expected since this secondary produced microspores of the formula $n, n + 9 \cdot 9, n + 9 \cdot 10$ and $n + 9 \cdot 9 - 9 \cdot 10$. The last class, $n + 9 \cdot 9 - 9 \cdot 10$, is deficient in the half $\cdot 10$ and aborts. The other three classes mature as pollen grains, and are all accounted for if we consider the $n + 9 \cdot 10$ to be relatively large in the pollen of Mutilated, especially in the test of figure 36. The correctness of this interpretation is indicated by the corresponding female inheritance of Mt, where records show that over a period of several years Mt plants have thrown in a total of 1293 plants, 76% normals, 14% Mt, 8% Ec, and 2% other unrelated 25-chromosome types. About a third of the 25-chromosome progeny of Mt is Ec.

Tests of Thistle ($2n + 10 \cdot 10$) are shown in figures 37 to 39. Here we must also look upon the $n + 10 \cdot 10$ pollen grains as the ones which fail to germinate. The $n + 9 \cdot 10$ grains apparently produce burst tubes which occur in much smaller proportions than in the other secondary. However, this condition is also borne out by the corresponding female inheritance. Thistle has thrown in a total of 1045 plants, 64% normals, 31% Th, 3% Ec, and 2% other unrelated types. Thus we see that while a high proportion of $n + 9 \cdot 10$ pollen tubes may be expected from tests of Mt there should be a low proportion in the tests of Th. This line of argument may be less successful with other chromosomal types since a number of factors may be involved affecting the type of chromosome disjunction in megaspore formation and the survival of the resulting extra chromosomal types. Moreover, it is uncertain how close the correspondence may be between the disjunction in megaspore formation, which is primarily responsible for $2n+1$ types in the progeny, and the disjunction in microspore formation, the results of which concern us in the present paper.

It may be concluded from our tests that none of the members of the Ec group may transmit the extra chromosome, that neither the $9 \cdot 10$, the $9 \cdot 9$, nor the $10 \cdot 10$ chromosome is transmissible as an extra.

Cocklebur and Its Secondary

Cocklebur (Ck) is another 25-chromosome primary whose extra chromosome belongs to the medium sized group M. This chromosome is designated as $11 \cdot 12$. Only one secondary is known, Wedge (Wd); its chromosome is $11 \cdot 11$. Since this primary and secondary have been made subjects of a special study (17), it will be unnecessary to describe a large series of these tests here in great detail. Figure 40 shows a typical test of the pollen of Ck and figure 41 that of Wd. It is evident that the $n + 11 \cdot 12$ pollen tubes may grow normally but at a slower rate than n tubes. When plants furnishing the pollen are growing under very unfavorable conditions many of the $n + 11 \cdot 12$ pollen tubes may burst or this pollen may even fail to germinate, but under moderately favorable cultural conditions one may

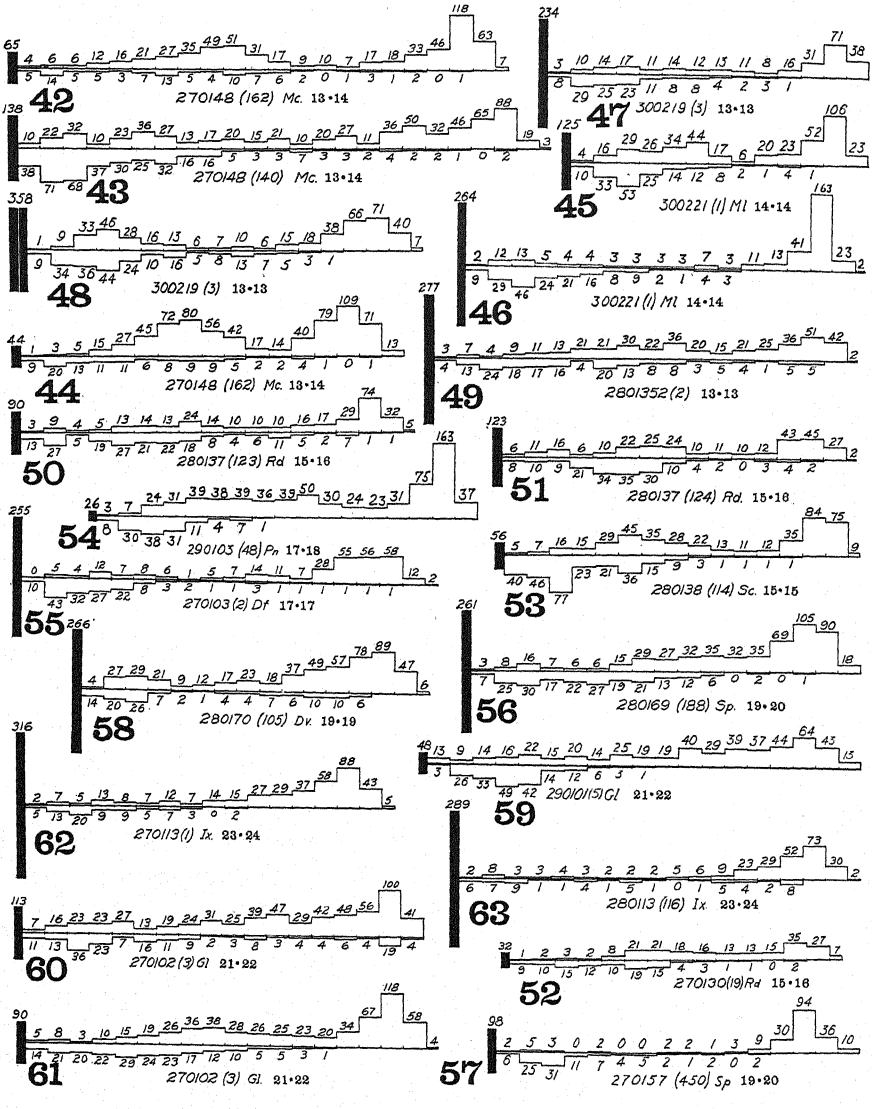
feel confident in obtaining a distribution of pollen tubes such as the one shown in figure 40. Wedge, which has microspores n, $n + 11 \cdot 11$, $n + 11 \cdot 12$ and $n + 11 \cdot 11 - 11 \cdot 12$, the latter forming aborted pollen, has the remaining three classes of pollen tubes shown in figure 41. The $n + 11 \cdot 11$ pollen tubes burst near the stigma, and the $n + 11 \cdot 12$ pollen tubes constitute a relatively small group midway between the n and $n + 11 \cdot 11$ tubes, corresponding to the small proportion of primaries thrown by this secondary through female inheritance (3). The transmission of the extra $11 \cdot 12$ chromosome has been verified by appropriate genetic tests (17).

The Microcarpic Group

Microcarpic (Mc) and its secondaries constitute a group involving another chromosome $13 \cdot 14$ of the size class M. One of its secondaries is Mealy ($2n + 14 \cdot 14$), and the other $2n + 13 \cdot 13$.

Microcarpic (Mc) gives very good bi-modal distribution curves for a limited period during the summer (usually about the middle of August) as shown in figures 42 and 44. There are two groups of pollen tubes differing only in their rate of growth—the forward group of n pollen tubes grows about twice as fast as the $n + 13 \cdot 14$ group. Earlier in the summer or late in August, the conditions for tests of Mc are not so favorable. Figure 43 shows a test of Mc in which conditions for pollen-tube growth were less favorable. Many pollen tubes have burst and the mode of distribution of $n + 13 \cdot 14$ pollen tubes shown in figures 42 and 44 has been broken up into several scattered groups. Under this condition one may expect a much less satisfactory transmission of the extra chromosome. At times the $n + 13 \cdot 14$ pollen grains germinate only partially or they may even fail entirely to germinate.

Mealy (Ml) is one of the secondaries of Microcarpic having as the doubled half $14 \cdot 14$. Tests of the growth of its pollen tubes are shown in figures 45 and 46. In the test of figure 45, about 81% of the pollen germinated. In figure 46, only about 64% of the pollen germinated. We may recognize the group of n pollen tubes in the forward mode constituting very nearly a third of the gametophytic population in both of these tests; hence the difference between these two tests comes from a variation in the proportions germinated which belong to the $n + 14 \cdot 14$ and $n + 13 \cdot 14$ groups combined. Figure 45 has about 46% of its germinated population not belonging to the n group while figure 46 has only 30%. The two tests were made in the latter part of August with only five days intervening. Whether the distinct second mode of figure 45 consists mostly of $n + 14 \cdot 14$ pollen tubes or $n + 13 \cdot 14$ cannot be determined with certainty; it is probable that both of these types of gametophytes may be found within this group and that one of them, probably the $n + 14 \cdot 14$, greatly predominates in figure 46, where the $n + 13 \cdot 14$ may be the part remaining largely as ungerminated pollen.



TEXT FIGS. 42–63. Explanation in text.

Tests of the other secondary $2n + 13 \cdot 13$, are shown in figures 47 to 49. Here the proportion of ungerminated pollen is high, from 33–38%. This ungerminated pollen plus a part of the burst pollen tubes probably represents the $n + 13 \cdot 13$ gametophytes. The n group includes a little less than a third of the total gametophyte population, and the pollen tubes remaining in the group between are probably the $n + 13 \cdot 14$ group including increments from the other two groups.

While the breeding behavior of the secondaries $14 \cdot 14$ and $13 \cdot 13$ as

shown by their female inheritance has not been determined from large numbers, it appears that they both throw their primary, Mc, in relatively high proportions. Thus we may at times expect a fairly large intermediate group of $n + 13 \cdot 14$ pollen tubes shown in some of the tests.

On the whole, it appears that under proper conditions the chromosome 13·14 may be pollen-transmitted as an extra, provided that the pollen germinates satisfactorily. The two secondaries seem to be non-transmissible: 13·13, because the pollen fails to germinate; 14·14, because it germinates poorly and gives rise to very slow-growing or burst tubes when germinated. We should probably expect that since Mc is pollen-transmitted, at least one of its secondaries should be, but this is not indicated by our tests thus far.

Reduced and its Secondary

Reduced (Rd) which involves another chromosome, 15·16, belongs to the size class m. One secondary Scalloped (Sc) has been discovered; its chromosome is designated as 15·15.

Figures 50 to 52 show tests of the pollen of Rd. Here there are two groups of pollen tubes, the *n* group in the forward mode and a retarded group of slow-growing, normal-appearing pollen tubes found in the region with many burst tubes. This class is somewhat variable in behavior. At times, the proportion of these tubes which burst may include nearly all the $n + 15 \cdot 16$ pollen tubes indicating practically no pollen transmission of the extra chromosome, but at other times the bursting is not so general so that this chromosome may be transmitted through the pollen as an extra. Under favorable conditions, one may expect a reasonable amount of pollen transmission.

A test of the secondary Scalloped (Sc) with the doubled half 15·15 as an extra is shown in figure 53. Here only 7.2% of the pollen remains ungerminated. The *n* pollen tubes in the advanced mode constitute about 29%, and the normal-appearing pollen tubes in the slow group, together with the group of burst tubes, make up 63.4% of the total population. Since the pollen germination was very high it is obvious that the latter groups must include the $n + 15 \cdot 15$ gametophytes and that these behave very nearly the same as the $n + 15 \cdot 16$ pollen tubes, some of which are also included in this group. The $n + 15 \cdot 15$ pollen tubes probably grow somewhat more slowly than the $n + 15 \cdot 16$. Both of these classes, whether carrying the extra chromosome of the primary or its secondary, are easily subject to bursting in their passage through the style, and the high proportions transmitted through the pollen in Ck might not be expected with Rd or Sc.

Poinsettia and its Secondary

Poinsettia (Pn) is a 25-chromosome plant involving one of the m chromosomes, designated as 17·18. One of its secondaries, Dwarf (Df) with the doubled half 17·17, has been known for some time.

Tests of Pn from field-grown plants were attempted over many years without satisfactory results. The diagrams obtained usually gave distributions with a backward skew and considerable ungerminated pollen. However, certain breeding evidence (not in Line 1) has indicated that Pn is sometimes transmitted by the pollen. The plants do not set capsules freely in the field, and for this reason we have resorted to tests of greenhouse plants. Figure 54 is a test made from the pollen of a greenhouse plant growing under favorable conditions. Here a distinct bimodal grouping of pollen tubes is recognizable and under these conditions the $n + 17 \cdot 18$ chromosome should be pollen-transmissible provided the amount of pollen used in the pollination is not excessive.

A test of Dwarf (Df), the only known secondary of Poinsettia, is shown in figure 55. This shows that very few if any of the $n + 17 \cdot 17$ pollen grains germinate. They have not germinated, though many tests have been made and repeated at various times. The portion of the pollen which has germinated aside from the n group may be the $n + 17 \cdot 18$ component in the pollen of this secondary, which seems likely, since there is a small group of slow-growing pollen tubes at the distance corresponding to the slow group in figure 54, but many of them have burst. Dwarf is, therefore, not pollen-transmitted. As in other secondaries, the $n + 17 \cdot 17 - 17 \cdot 18$ microspores form aborted pollen.

Spinach and its Secondary

Spinach (Sp) is one of the primaries whose chromosome $19 \cdot 20$ belongs to the size class m. Figures 56 and 57 show pollen-tube distributions from tests of Sp. Since Sp is a primary we may expect it to produce only two kinds of gametophytes, n and $n + 19 \cdot 20$. Neither the burst pollen tubes alone, nor the ungerminated pollen grains on the stigma taken alone, could account for enough gametophytes to balance the n pollen tubes, but the two groups balance fairly well when ungerminated and burst pollen tubes are considered together. Our tests, therefore, indicate that the $n + 19 \cdot 20$ gametophytes may germinate in part and remain ungerminated in part.

Divergent (Dv) is the only known secondary of Spinach and has the $19 \cdot 19$ as an extra chromosome. A test of its pollen-tube growth is shown in figure 58. These tests seem to resemble Sp more or less, but have a higher proportion of ungerminated pollen. Neither Spinach nor its secondary Dv appear to be pollen-transmissible.

Globe

Globe (Gl) has an extra the chromosome $21 \cdot 22$ of the size class S. It has no known secondary. Figures 59–61 show tests of the pollen of Globes. In figure 61 the $n + 21 \cdot 22$ pollen tubes are growing slowly in a group which has a wide spread and has grown only about half the distance of the n tubes. A large portion of the $n + 21 \cdot 22$ pollen tubes have grown about

two-thirds as far as the *n* tubes in figures 59 and 60. There are many pollen tubes which have burst or remain as extremely slow-growing tubes in all of the tests, but there is usually a fairly large group of $n + 21 \cdot 22$ tubes which may be expected to reach the ovary to accomplish fertilization.

Globe is pollen-transmissible under favorable conditions. The study of pollen transmission of Globes has been the subject of special study carried on over many years. With restricted pollinations we have obtained as high as 30% transmission for the whole capsule in individual cases, though the average for all attempts is far below this figure. The results of our genetic tests of the pollen transmission of Gl, too lengthy to be included here, will be reported elsewhere. It appears that the $n + 21 \cdot 22$ pollen tubes may at times grow at more than two-thirds the rate of the *n* pollen tubes, at times they grow at a slower rate and a considerable proportion of them may burst within the style.

Ilex

Ilex is a 25-chromosome plant which has as an extra the smallest chromosome, s, designated $23 \cdot 24$. No secondary of Ilex has been recognized. Figures 62 and 63 show tests of the pollen of Ilex. The plant is very late in flowering when growing in the field, and usually half the pollen remains ungerminated as shown in the figures. The smallest chromosome $23 \cdot 24$ is, therefore, not transmitted by the pollen as an extra in field-grown plants because these pollen grains fail to germinate. No tests of the pollen of greenhouse Ix plants have been made.

Discussion

The various chromosomes of *Datura Stramonium* Line 1 *A* may be classified and arranged according to their pollen-transmissibility as extra chromosomes. Judging from the pollen-tube growth of the primaries the approximate order of their ease of transmissibility is: Ck, Mc, Gl, Rd, and Pn. For all of them the conditions of pollen-tube growth would have to be favorable, though a wider range of favorable conditions would be available for Ck than for the rest. Pollen transmission of Pn could be obtained only by using the pollen of greenhouse plants.

We may list Gs in a second category of primaries in which pollen transmission of the extra chromosome might be obtained occasionally, but only in a very small percentage of the progeny, when growing under the conditions most favorable to pollen-tube growth. Elongate might possibly belong in this category but the low viability would probably prevent the El zygotes which might arise through pollen transmission from appearing in the progeny. For this reason we include El in the non-transmissible group.

The following primaries are considered non-transmissible because the pollen tubes which carry the extra chromosome burst within the style: Rl,

El, Bk, Ec, Sp; and Ix should be included because the $n + 23 \cdot 24$ pollen grains fail to germinate. Under unfavorable conditions those of Rl, El, Bk, and Sp, as well as most of those enumerated among the pollen-transmissible group, may fail to germinate.

Among the secondaries, pollen transmission of the doubled half chromosome may be expected in Sg, At, and possibly in Sc. In many experiments in which trisomic types are desired from pollen-transmission, Sg could be substituted for Rl, and At for Bk, while Sc offers no advantage over Rd, which is probably more easily pollen-transmitted than its secondary. The conditions for Ml are at present somewhat uncertain. Two chromosomes are, therefore, added to the list which may be pollen-transmitted as extras either through their primaries or their secondaries.

The remaining group of secondaries, Py, St, Mt, Th, Df, Dv, and $13 \cdot 13$ are non-transmissible because the $n + 1$ pollen grains fail to germinate; Sm, Un, Wd and Ml (?) because the portion which may sometimes germinate, bursts within the style. Most of these secondaries, with the exception of Wd, give a very poor germination of the pollen grains which contain the extra chromosome.

The rates of growth of the 13-chromosome pollen tubes also vary considerably. When at their best the pollen tubes with $n + 21 \cdot 22$ chromosome of Gl seem to grow from 2/3 to 3/4 of the rate of the n tubes. The $n + 2 \cdot 2$ pollen tubes of the secondary Sg grow at about 2/3 of the rate of n tubes. The 13-chromosome gametophytes of Ck, Mc, Rd, Pn, El, and Gs grow at a rate somewhere between 3/5 to 2/5 as fast as n, and the $n + 6 \cdot 6$ pollen tubes of the secondary At grow at about 1/3 of the rate of the n tubes. Of course, these approximate rates of growth should be based on the positions of the 13-chromosome pollen tubes which are still growing, and not on the average position of their entire groups which would include the burst tubes.

Another consideration which is involved with genetic tests of pollen transmission is the question of viability (3). Some of the 25-chromosome plants have a low rate of survival in their female inheritance as shown in the progenies obtained from selfed capsules. Thus Ix stands highest among the primaries, being obtained in nearly 34% of its progeny, Pn 30%, and El stands relatively low at about 10%. There is a differential elimination among the female gametophytes, ovules, seeds, and seedlings, which tends to prevent the formation of, or to destroy, the zygote with extra chromosomes, and the relative viability of a type may be taken as an index of the probability of the survival of a 25-chromosome zygote should it originate by pollen-transmission or otherwise. When their origin is from pollen transmission, a plant such as Pn would stand a much greater chance in its survival during interovular selection and the other eliminative processes than Rd (19%) or Sg (20%). Thus while our tests indicate that El might appear to transmit about as high a proportion of 13-chromosome pollen tubes to the ovary as Gs, its low viability would greatly lower the proportion of these which might survive in the seed progeny. For this reason, we

consider Gs more likely to be successfully pollen-transmitted than El, and even Gs is very questionable.

It is possible that we have, for practical purposes, somewhat overestimated the pollen-transmissibility of some of the extra chromosomes. It should, therefore, again be pointed out that the conditions under which our laboratory tests of pollen-tube growth have been made are quite ideal. We feel assured that such conditions would be relatively infrequent in a garden culture, but they may be obtained and controlled for potted greenhouse plants, as in the experiments with Ck (17). We have had some success in transmitting Gl and Sg through the pollen by operating on plants growing in the field (15).

One of the chief obstacles to be overcome in the pollen transmission of the extra chromosome is the great preponderance of *n* pollen tubes which arrive at the ovary first and may fertilize all or nearly all of the eggs, leaving few ovules to be entered by late-arriving pollen tubes.

There are several practical pollination methods which may be used to overcome this difficulty. If the pollinations are restricted to about 500 pollen grains, the *n* pollen tubes cannot monopolize all of the ovules and any slow-growing $n + 1$ pollen tubes which arrive in the ovary later may fertilize some of the remaining ovules. These are usually situated in the lower part of the seed capsule (17). By another method the *n* pollen tubes may be excised and discarded after a period of growth in the pistils of cut flowers in the laboratory, and the style with short tubes attached to the lower part of the style of an unpollinated flower (18). We have obtained 25-chromosome offspring by the use of both of these methods.

Our investigations (18) on spliced styles in field-grown plants have also brought out the fact that at least 20–30 pollen tubes should enter the ovary in order to ensure the setting of a fruit. Thus, we should have to have a slow-growing group of pollen tubes as large as 20 or more. For Gs, at least, there is some question as to whether there would be as many as 20–30 pollen tubes which might survive bursting and successfully cross the place of splicing. These studies of pollen-tube growth in 25-chromosome plants have served to indicate which of the primaries or secondaries might be used for restricted pollinations or for style-splicing operations with a reasonable probability of success.

Origin of 25-chromosomal Types

Aside from these more practical considerations concerning the pollen-transmissibility of extra chromosomes there is the question of the origin of chromosomal types and their fate under natural conditions. If nondisjunction occurs in megasporogenesis, the extra chromosome has the chance of survival through female inheritance somewhat limited since the female transmission of extra chromosome plants is not in the chance proportion of 50% but more nearly in the propotion of 30% for the most viable 25-chromosome zygotes with various smaller proportions for others down

to 3% or less. The interovular elimination of 25-chromosome zygotes or the female gametophytes which could produce these zygotes occurs again and again in every life cycle. Interovular selection and other eliminative processes therefore greatly reduce the proportions of extra-chromosomal plants in the progeny on the female side, where conditions for their survival are most favorable.

Gametophytic selection during pollen-tube growth is far more severe, eliminating the extra chromosome of 7 primaries before the fertilization stage is reached, and sifting out the extra chromosomes which would produce the remaining types in high proportions. For five of the 12 chromosomes, there might be an occasional 25-chromosome plant which could survive and owe its origin to non-disjunction in microsporogenesis.

There is the possibility of a slight difference between a 13-chromosome microspore derived from non-disjunction in a diploid plant and one coming from microsporogenesis in a 25-chromosome anther. The former has its initial cytoplasm derived from a diploid pollen mother cell, while the latter has its initial cytoplasm derived from a 25-chromosome pollen mother cell. As we have pointed out elsewhere (16), the volume of the microspore enlarges about 2400% and its nucleus divides at least once in the process of maturing as a pollen grain, but in one case the environment in which this happens is a diploid anther, in the other it takes place within the 25-chromosome anther where the metabolism of the surrounding tapetal cells may not be the same. The question of whether these differences could be responsible for a difference in pollen-tube growth remains unanswered by these experiments, since we are only concerned with pollen which had matured in the anthers of a 25-chromosome plant. We can only state our belief that the effect of the cytoplasm contributed from the microspore mother cell is relatively insignificant, since there is this prolonged intervening period of development before a pollen grain is matured during which the influence of the contained extra chromosome cannot be eliminated in either case, and that the adverse influence of the tapetum and the surrounding tissues of a 25-chromosome plant, if any, is probably not as great as the difference which may be found in the nutritive conditions existing at times among different plants, or among different flowers on the same plant.

The role of the mechanism of gametophytic selection (during pollen-tube growth) is that of imposing a severe censorship upon any new chromosomal types. Deficiencies due to the absence of a half or more of any chromosome are eliminated within the anther, and give rise to aborted pollen grains. Additions of one or more chromosomes are usually not eliminated until after pollination, where they tend to interfere with the proper functioning of the male gametophyte at germination or during pollen-tube growth. Thus it has been found that in self pollinations and in routine crosses the male transmission of extra chromosomes is practically negligible (2, 3).

On the whole, the effect of these internal selective processes is to censor any chromosomal innovations; to sift out nearly all chromosomal types and perpetuate only the diploid condition so that from 67–97% of the progeny of the various 25-chromosome plants result in $2n$ offspring.

Summary

1. The necessary conditions for making tests of pollen-tube growth in $2n$ and $2n + 1$ plants are described.

2. Comparable tests of pollen-tube growth employing the pollen of $2n + 1$ plants on styles of $2n$ plants are described and compared for 12 primaries and 14 secondaries.

3. The interference of extra chromosomes with pollen-tube growth differs considerably for the various chromosomes, and is independent of the size of the extra chromosomes.

4. The causes of non-transmission of extra chromosomes through the pollen may be classified under: (1) non-germination of the $n + 1$ pollen; (2) cessation of growth with swelling and bursting of $n + 1$ pollen tubes within the style; (3) conditions more or less intermediate between (1) and (2); (4) slow growth of $n + 1$ pollen tubes; (5) conditions intermediate between (4) and (1), or (4) and (2).

5. The tests indicate that six of the twelve chromosomes of primaries are not pollen-transmissible as extras under any known conditions, one other chromosome might possibly be transmitted under very exceptional conditions, and five of the chromosomes of primaries may be transmitted as extras.

6. The pollen-transmissibility of a number of the secondaries increases the list to 7 or 8 chromosomes which may be transmitted either directly through their primaries or indirectly through secondaries.

7. The slow-growing pollen tubes with extra chromosomes range in growth rate between 1/3 and 3/4 of the rate of n pollen tubes.

8. Gametophytic selection modifies greatly the proportions of the $2n + 1$ types obtained in male back-crosses, and may be controlled through a wide range by several methods: (*a*) restricted pollinations; (*b*) selection of seeds from lower half of seed capsule; (*c*) splicing of styles after excising and discarding the longer n tubes.

9. There is a possibility that among the pollen grains arising from non-disjunction in microsporogenesis, the primaries Gl, Ck, Mc, Rd, and Pn might occasionally give rise to $2n + 1$ zygotes which might appear as spontaneous mutants.

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A NEW *PLAGIOCHASMA* FROM TEXAS[1]

ALEXANDER W. EVANS

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Two species of *Plagiochasma*, *P. rupestre* (Forst.) Steph. and *P. Wrightii* Sulliv., have been reported from the United States, *P. rupestre* being known from New Mexico and Arizona and *P. Wrightii* from Oklahoma, Texas, and Arizona.[2] It is now possible to add a third species to this short list. This species, which is apparently undescribed, is based on material collected by Professor F. McAllister at Hamilton's Pool, about thirty miles west of Austin, Texas. It may be characterized as follows:—

Plagiochasma cuneatum sp. nov.

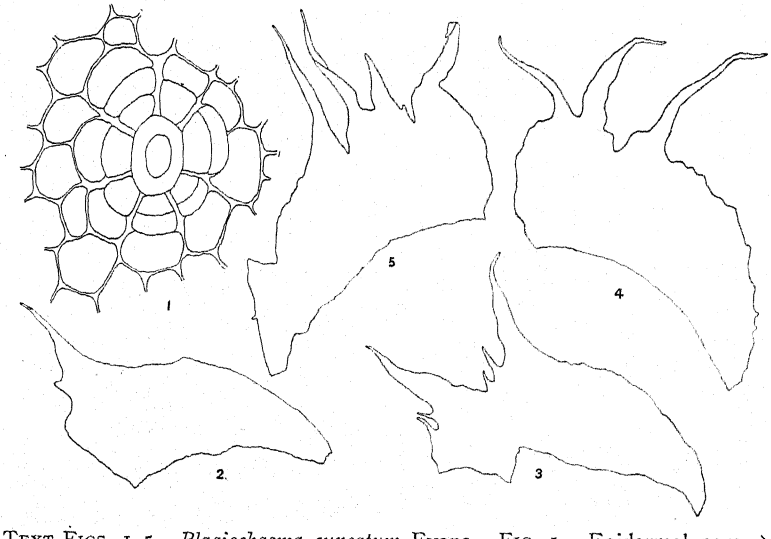
Frons ligulata, 0.75–1 cm. longa, 3–4 mm. lata, viridis, subtus purpurea, plana sed siccando involuta, rare dichotoma, ramos ventrales paucos proferens, articulata, articulis cuneatis; cellulae epidermidis tenerae, trigonis parvis; pori magni, elevati, a cellulis 4–10 seriatis circumdati, parietibus radiatis incrassatis; squamae ventrales lunulatae, purpureae, appendiculis saepe 2–3, subulatis, longe acuminatis: flores monoici, terminales in articulis frondis; carpocephala longe pedunculata, bi- vel trilobata, lobis brevibus, plerumque rotundatis; sporae 95–110 μ, fusco-flavae, grosse reticulatae; elateres 200 μ longi, fusco-rufi, bi- vel trispiri.

Thallus bright green above with a narrow purple border, deep purple below, at least in the older parts, and often showing a greenish metallic iridescence when dry, plane or nearly so above when moist, the wings strongly incurved when dry and more or less concealing the upper surface, strap-shaped, rarely forking, occasionally branching ventrally, on fruiting plants divided into joints formed by apical innovations, mostly 0.75–1 cm. long and 3–4 mm. wide, the thickness about one-fifth as great as the width, slightly crispate along the margin; epidermis colorless, composed of thin-walled cells, averaging about 21 μ in diameter, with small but distinct, occasionally coalescent trigones having straight or slightly concave sides; cuticle very finely verruculose; pores conspicuous and distinctly elevated, surrounded by four to ten, usually seven or eight, radiating series of cells with two or three cells in each series, radial walls distinctly thickened; aeriferous layer about five-eighths the thickness of the thallus, the upper half much greener than the lower and showing air-spaces about twice as wide as the lamellae; cells containing oil-bodies occasionally present among the green cells as well as in the compact ventral tissue but lacking in the epidermis; ventral scales lunulate, slightly overlapping and covering over the ventral surface of the thallus, irregularly sinuate or sinuate-dentate along the margin, composed of irregular cells with strongly pigmented, almost opaque walls, the cells containing oil-bodies shorter than the others and with unpigmented walls; scale-appendages one to four (usually two or

[1] Contribution from the Osborn Botanical Laboratory, Yale University.

[2] See **Evans**, N. Am. Flora **14**: 41, 42. 1923.

three) in number, mostly purple but sometimes becoming bleached toward the tips, subulate to narrowly subulate, long-acuminate, the apex usually formed by a row of three to five cells, margins entire or rarely with one or more short cilium-like teeth: inflorescence normally monoicous: inflorescence of each sex at first terminal, the thallus (almost always) continuing its growth by means of an apical innovation and thus acquiring a jointed appearance, the boundaries between the joints cuneate and pointing backwards; peduncle of carpocephalum mostly 3–5 mm. long, deep purple on the outside, colorless within; carpocephalum with a low apical depression, mostly two- or three-lobed, the lobes bluntly pointed or rounded; scales of carpocephalum linear-lanceolate, entire; spores 95–110 μ in diameter, deep golden brown (in transmitted light), covered with a coarse reticulum, the



TEXT FIGS. 1–5. *Plagiochasma cuneatum* Evans. FIG. 1. Epidermal pore, × 300. FIGS. 2–5. Ventral scales, × 27. The figures were all drawn from the type-material.

spherical face usually with eleven to thirteen meshes and each triangular face with four or five, outermost layer of spore-wall minutely verruculose, the verruculae in the form of rods or short ridges, the latter sometimes more or less coalescent and forming a very delicate and often incomplete network; elaters mostly 200–300 μ long and 10–12 μ in diameter, reddish brown, gradually attenuate, spirals two or three, more or less connected by longitudinal bands of thickening, the thin places in the wall somewhat pigmented.

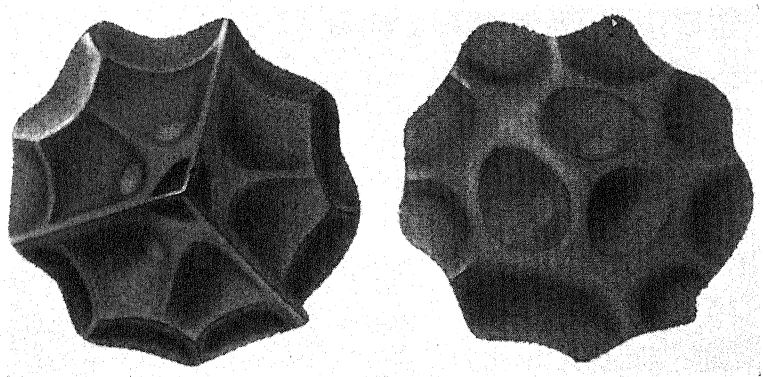
On soil, Hamilton's Pool, Texas, *F. McAllister*, February, 1931. Type in the herbarium of Yale University.

The epidermal pores of *P. cuneatum* (text fig. 1) are more conspicuous than in many species of the genus and, under the lens, stand out as white dots in a green background. The cells surrounding the opening are usually in seven or eight radiating rows, with two or three cells in each row. In *P. Wrightii* and *P. Landii* Evans, with which *P. cuneatum* might perhaps be confused, the number of radiating rows is usually six. The pores of *P.*

cuneatum thus tend to be more complex than in these two species. They resemble, in fact, the large pores of *P. intermedium* Lindenb. & Gottsche, in which the number of radiating rows is usually eight, and the resemblance is increased by the pronounced thickening of the radial walls.

The appendages of the ventral scales (text figs. 2–5) exhibit a number of distinctive features. In most species of *Plagiochasma* the number of appendages is usually one or two, but in *P. cuneatum* there are usually two or three appendages, although the number varies from one to four. The appendages of the youngest scales bend back over the growing-point and are usually deeply pigmented with purple. Their slender, subulate form, tapering to a long point, will at once distinguish them from the ovate and abruptly pointed appendages of *P. Wrightii*. They resemble, in fact, the appendages of *P. Landii*, but these are usually borne singly and tend to be much broader.

The spores of *P. cuneatum* (text figs. 6 and 7) are deeply pigmented and



6 7

TEXT FIGS. 6 and 7. *Plagiochasma cuneatum* Evans. FIG. 6. Spore, inner view, showing the three plane faces, × 470. FIG. 7. Spore, outer view, showing the spherical face, × 470. The figures were both drawn from the type-material.

show the coarse reticulation which is characteristic of the genus as a whole. The ridges of the reticulum, which represent folds of the outer wall-layers, are rather broad at the base and thin out to rounded or fairly sharp edges, the latter being found along the wing of the spore and along the three converging lines bounding the triangular faces. The meshes, in consequence, expand outward from a relatively small base. On each of the three triangular faces there are usually four meshes, the three peripheral occupying the base of the triangle and the fourth, or apical, mesh the apex. This fourth mesh, however, is often subdivided by a broad low ridge into two meshes. When the division is complete, the bases of the meshes are visible as two small elliptical areas; when the division is incomplete, the elliptical areas sometimes coalesce to an hour-glass-shaped area; when there is no

division the base of the fourth triangle appears as a single elliptical area. The spore illustrated (text fig. 6) shows a complete division of each apical mesh and brings out the contrast in size between the base of a mesh and its outer boundary. The bases of the peripheral meshes are not visible, being concealed by ridges. The meshes of the spherical face of the spore form a central group surrounded by a peripheral series. In its most regular development the central group, as shown in text figure 7, includes three meshes, but four are sometimes present; the peripheral series usually includes nine meshes, but this number may be reduced. The spore illustrated, for example, shows only eight peripheral meshes. The peripheral meshes of the spherical face do not correspond with those of the triangular faces but alternate with them. This becomes evident if the two figures are compared. In text figure 6 the blunt projections at the circumference correspond with the ridges which form the lateral boundaries of the peripheral meshes, while in text figure 7 the projections are midway between these ridges. This is true except in the case of the large mesh on the lower side of the figure, where two projections are present. The three angles of the spherical face, which correspond with the ridges separating the three triangular faces, are scarcely distinguishable by their form from the other projections. Sometimes, however, the angles are marked by minute perforations through the outer wall-layer, and a perforation of this character is shown on the upper side of the figure.

The spores of *P. cuneatum*, which average about 100 μ in diameter, are considerably larger than those of *P. Wrightii* and *P. Landii*, which average only 75–80 μ. They are also more deeply pigmented, and the same thing is true of the elaters.

A remarkable feature of *P. cuneatum*, which will at once distinguish the species from all the other American representatives of the genus, is the division of the fertile thallus into a series of joints. Each joint tapers to a point at the posterior boundary and shows a similar indentation at the anterior boundary. At the bottom of the indentation an inflorescence, either male or female, is situated. This condition is apparently caused by a temporary cessation of growth induced by the development of each inflorescence. When growth is resumed, perhaps by means of the old apical cell, perhaps by the differentiation of a new apical cell, a joint is formed. In some cases the pointed end of a joint, although completely filling the indentation of the previous joint, has no organic connection with it except at the very narrow base. Often, however, there is a more extensive coalescence between two joints, the region of coalescence being marked by a shallow groove. The groove may extend to the margin of the thallus or come to an end at any intermediate point. In extreme cases no groove is present, and the tissues on both sides of the inflorescence are perfectly continuous. This is, of course, the usual condition in *Plagiochasma*.

A jointed thallus in the present genus is by no means unusual and was

noted many years ago by Nees von Esenbeck[3] in *P. cordatum* Lehm. & Lindenb. of India and *P. peruvianum* Nees & Mont. of Bolivia and Peru. In many cases the joints have nothing to do with inflorescences and occur in sterile thalli, but Goebel has figured a plant of *P. cordatum* in which a joint follows a male inflorescence.[4] The phenomenon, in fact, seems to be more frequently associated with male inflorescences than with female, and only one species has heretofore been described in which a joint regularly follows a female inflorescence. This species is the xerophilous *P. articulatum* Kashyap of India.[5] It was based on material collected in the Himalayas but has been recorded also from lower altitudes. *P. articulatum* is known to the writer from specimens collected in the Mussorie region by R. S. Copra and distributed by Verdoorn.[6] These specimens, together with Kashyap's descriptions and figures, show that *P. cuneatum* and *P. articulatum* are closely related but not synonymous. Aside from their jointed thalli they agree in having similar epidermal cells and pores and similar ventral scales, the latter being characterized in both by the possession of slender, pointed appendages, usually two or three in number. The scales of *P. articulatum*, however, are less deeply pigmented than those of *P. cuneatum*, the cells containing oil-bodies are less numerous, and the appendages are more abruptly pointed. The spores yield even more important differences. Those of *P. articulatum* measure only 60–80 μ in diameter, their color is a pale yellowish, and they are bordered by a distinct wing. According to Kashyap the surface is reticulate-lamellate, but the reticulum is poorly developed and indistinct in Copra's specimens.

The figures of the spores illustrating the present paper were prepared by Mrs. Rose Meyrowitz Freeman of Baltimore, and the writer would here express to her his sincere thanks. The figures were drawn from microscopic mounts supplemented by plasticine models.

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[3] Naturg. der Europ. Leberm. 4: 36. 1838.

[4] Organographie, 2nd ed. *f. 669*. 1915.

[5] New Phytol. 13: 320. *f. 8*. 1914; Liverworts of the western Himalayas and the Punjab Plain 75. *Pl. 16, f. 1–3*. 1929.

[6] Hep. Sel. et Crit. No. 4. 1930.

LIGHT IN RELATION TO THE SEED GERMINATION OF *MIMULUS RINGENS* L.[1]

SELAR S. HUTCHINGS

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INTRODUCTION

In the vast majority of the seed plants, all that is necessary to induce germination in mature seeds is to expose them to suitable conditions of moisture, temperature, and air. With some seeds, however, exposure to these so-called "normal conditions for germination" is insufficient. In particular, as has long been known, there are certain seeds in which germination will take place only in the presence of light, and there are many others which germinate much better in the light than in the dark.

Attention apparently was first called to this phenomenon of light sensitiveness in seeds by Caspary (1860). The conclusions which he drew, however, were disputed by Nobbe (1876, 1882), who maintained that light has little or no effect on germination, and by Pauchon (1880), who supported the views of Nobbe. But Caspary's findings were upheld by Wiesner (1878), Stebler (1881), and Cieslar (1883); and subsequent work by various investigators has only served to increase the known number of light-sensitive seeds. The most extensive contributions on this subject have been made by Kinzel (1907, et seq.), whose studies, carried on over a period of twenty years, have considered the effects of both light and temperature conditions on seed germination. As a result of his experiments with fully 1200 different plants, a large number of light-sensitive seeds have been brought to attention.

The present investigations have to do with the germination of *Mimulus ringens* L., the monkey flower, a plant native to the eastern United States, where it is widely distributed in wet grounds. Kinzel (1920) has already recorded the failure of the European *Mimulus luteus* L. to germinate in the absence of light. His observations for this species covered a period of three years, at the expiration of which, upon being transferred from darkness to sunlight, the seeds showed a prompt germination of 99 percent. A similar dependence on light for germination in *Mimulus ringens* was first noted by Mitchell (1926); and her results have been confirmed by Diller (1930), whose experiments "show conclusively that light is a limiting factor in the germination of this species."

In the studies here described the author has undertaken to ascertain, somewhat more exactly than has heretofore been done, the extent to which

[1] Contribution from the Osborn Botanical Laboratory, Yale University.

light acts as a limiting factor in the germination of *Mimulus ringens*, and whether or not the influence of light in this respect may be replaced by that of some other environmental factor.

Materials and Methods

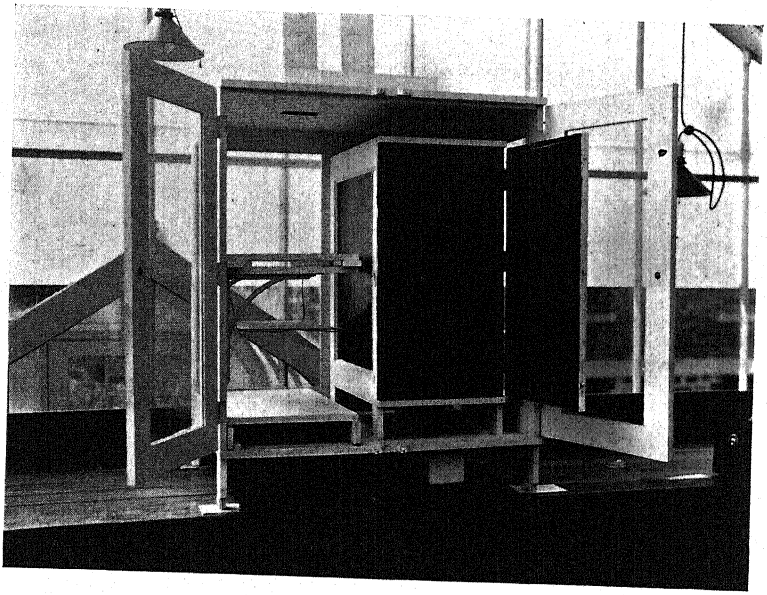
The seeds used in the present studies were collected in the vicinity of New Haven, Connecticut, during the latter part of September, 1930. After being dried and cleaned, they were placed in a bottle and stored dry at a temperature about 10° to 15° C. For purposes of experiment the seeds were counted out in lots of 200, as needed. At the outset some difficulty was experienced in counting the seeds, due to their exceedingly small size; but this was overcome by the following procedure. The seeds to be counted were first scattered loosely on a sheet of paper which had been ruled into one-eighth-inch squares. Then, beginning with a vertical row of squares on the left and working toward the right, all the seeds were counted in a series of squares until the required number had been secured, after which the surplus seeds were scraped off with a knife and the lot recounted. By this method, and with the aid of a reading glass, it was possible to count off successive lots of seed with both speed and accuracy.

The germination experiments were carried out in petri dishes. These were first thoroughly washed with soap and water, rinsed, and dried, after which four filter papers were placed in each and the whole steam-sterilized. The seeds to be germinated were then carefully scattered over the surface of the filter paper, this being first moistened with distilled water. More water was added during the course of the experiment, whenever needed, to maintain proper moisture conditions. No difficulty was experienced with molds, except in one case where the seeds had been allowed to after-ripen in an ice box for five months.

The experiments here described were conducted, for the most part, in the greenhouses of the Marsh Botanical Garden, Yale University, between the months of September, 1930 and May, 1931, inclusive. For contrasting the germination behavior of seeds in light and darkness, use was made primarily of the "light and dark box" described by Diller (1930). This box (text fig. 1) comprises two chambers, one open to light and the other not, which are so constructed and arranged that environmental conditions within the two are essentially identical in every respect except illumination. The "dark box," made of copper, is housed within a large glass case of which it occupies about one-half, the unoccupied half comprising the "light box." Throughout the course of the present experiments the entire case was screened from direct sunlight by a single thickness of fine cheesecloth, thus obviating any possible effects of differential heating and at the same time creating diffused light conditions in the light box, which accordingly is hereafter designated the "diffused light box."

In certain experiments use was made of a glass electric oven which was

set up alongside the "diffused light and dark box" and, like the latter, screened from direct sunlight by cheesecloth. Temperatures in this "diffused light oven" were maintained at 32° C. during the daytime; otherwise the conditions were similar to those in the "diffused light box." "Dark oven" conditions were obtained by wrapping the petri dishes in



TEXT FIG. 1. "Light and dark box" used for germination of seeds in light and darkness.

black paper and placing them in the "diffused light oven." Various other equipment used will be described in connection with the experiments concerned.

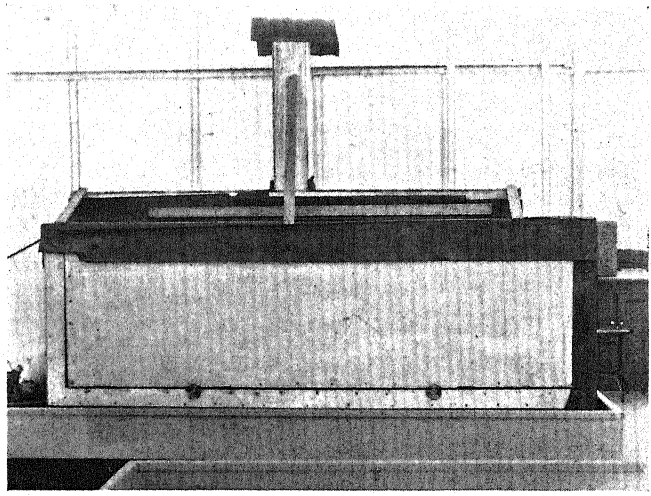
GERMINATION BEHAVIOR IN DIRECT SUNLIGHT, DIFFUSED LIGHT, AND DARKNESS

As a preliminary experiment, several lots of 200 seeds were placed in each of the following positions: (1) greenhouse bench, exposed to direct sunlight; (2) diffused light box; (3) dark box. This experiment served to bring out a number of fundamental facts, as follows: (1) Freshly collected *Mimulus* seeds have practically a 100 percent germination capacity or viability. This high viability, in fact, is still retained by seeds which have been kept for many months in dry storage. (2) Under favorable conditions, germination is relatively rapid. Ordinarily the seeds show no visible change before the eighth day, when about 49 percent germination takes place. For the following three days the "normal" total germination figures are approximately 88, 97, and 100, with germination complete at the end of the 11th day. (3) These seeds are markedly light-sensitive. No germination what-

ever takes place in darkness, while in diffused light the germination is greatly retarded, the average total germination here being 29.2 and 42.2 percent at the end of 8 days and 3 weeks respectively, with no further increase at the end of 6 months; and this in comparison with 49 percent germination in direct sunlight within 8 days with complete (100 percent) germination in 11 days. (4) Absence of light, however, does not impair germination capacity. The transfer of all ungerminated seeds from the diffused light and dark boxes to bright sunlight, after a period of 6 months, resulted in 100 and 99.2 percent germination within 5 days.

Effect of Light Intensity on Germination

For this series of experiments use was made of a wooden box two feet wide by two high by eight long, light-proof but well ventilated and with a door extending horizontally across the front (text fig. 2). The box was painted



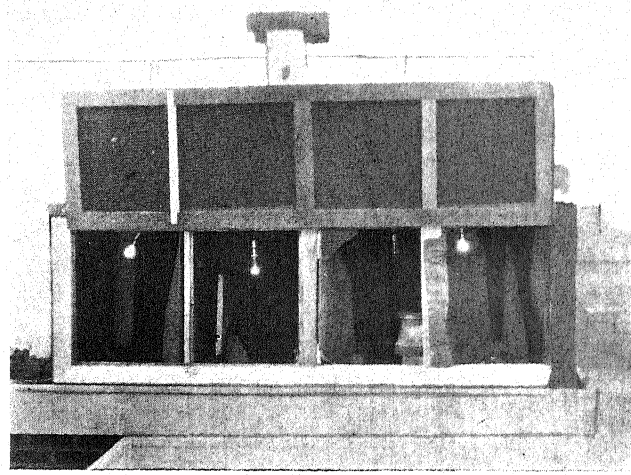
Text Fig. 2. "Differential illumination box" used in determining effect of differences in light intensity on germination; outside view.

white on the outside, to avoid overheating, and black within, to prevent selective absorption of light. The interior of the box (text fig. 3) was divided crosswise into four compartments which were equipped, at the top, with electric light bulbs of different light intensities. The light intensity in each compartment was measured by placing a foot candle meter at the surface of the petri dishes containing the seeds. For the various compartments the following light intensities, measured in foot candles, were thus obtained: 6.0, 3.5, 1.5, 0.64, and 0.16. (The fifth figure was secured by varying the distance of the dish from the light in one compartment.)

Table 1 shows the behavior of seeds exposed to these five light intensities

TABLE 1. *Germination Percentage of Mimulus Seeds under Various Foot Candle Light Intensities over Various Periods of Time*

Days	6.00 F.C.	3.50 F.C.	1.50 F.C.	0.64 F.C.	0.16 F.C.
0	0	0	0	0	0
5	52.5	22.0	1.0	—	—
7	60.0	25.5	2.5	—	—
10	64.5	32.5	3.0	—	—
15	69.5	34.0	10.5	—	—
34	72.5	34.5	11.5	—	—
47	75.5	—	—	—	—
62	—	—	—	—	—
Totals	75.5	34.5	11.5	0	0



TEXT FIG. 3. Inside view of "differential illumination box."

continuously for a period of more than two months. At the two lower light intensities there was no germination. At the three higher intensities the total germination was roughly proportional to the intensity of the light to which the seeds were exposed, viz., 75.5 percent at 6.0 F.C., 34.5 percent at 3.5 F.C., and 11.5 percent at 1.5 F.C. These results are in harmony with those of Lakshmana (1925) for *Lythrum salicaria* L. At the end of this experiment, all ungerminated seeds were transferred to bright sunlight, where every lot showed a germination capacity in excess of 92 percent.

EFFECT OF SHORT EXPOSURES TO LIGHT ON GERMINATION

According to various investigators, germination may be induced in many light-sensitive seeds by a very brief exposure to light. Thus Raciborski (1900) found that tobacco seeds, after being soaked in water, germinate much more readily if exposed to daylight for from one to five hours than if kept in the dark. In the same way, Lehmann (1918), by exposing

fully imbibed seeds of *Lythrum salicaria* for one-tenth of a second to a 730 candle-power lamp, at a distance of one meter, was able to secure 50 percent germination in the dark within 24 hours, whereas but very few of the seeds germinated in the dark without such exposure. In seeds of this description light would seem to act merely as an initial stimulus in germination: in the opinion of Lehmann, it serves to institute certain chemical changes.

In the case of *Mimulus ringens*, three series of "minimum exposure" experiments were carried out. In the first, which was continued over a period of two months, different lots of seed sown on moist filter paper were exposed to sunlight for different lengths of time daily throughout the course of the experiment, namely for 5, 10, 15, 30, and 45 minutes; and for 1, 2, and 3 hours, respectively. The results showed (1) no germination in two months, following exposures of one hour or less; (2) slow and incomplete germination following a daily exposure of 2 hours—total germination 6 percent at the end of two, and 89 percent at the end of six weeks; (3) somewhat more rapid but still greatly retarded germination following a daily exposure of 3 hours—total germination 25 percent at the end of two, and 97.5 percent at the end of six weeks. Upon transfer to full sunlight at the conclusion of the experiment, all the above lots of seeds showed a total germination in excess of 94 percent.

In the second series of "minimum exposure" experiments, seeds on moist filter paper were exposed to ordinary daylight conditions continuously for varying lengths of time, namely 1, 2, 3, 4, 5, 6, and 7 days, respectively, thereafter being placed in the dark box to germinate. The results, in brief, were as follows. (1) Seeds exposed to light for three days or less failed to germinate within the thirty days over which the experiment was continued. (2) Seeds exposed to light for four days or more germinated more or less in proportion to the length of the exposure. Thus those which had been exposed 4, 5, 6, and 7 days, in order, germinated 1.0, 5.5, 8.5, and 14.5 percent respectively on the eighth day. They showed total germinations of 2.5, 8.0, 11.0, and 16 percent respectively on the ninth day and of 4.5, 9.5, 12.0 and 17.5 percent respectively on the eleventh day. (3) None of the seeds showed any signs of further germination activity after the eleventh day, with the exception of the seeds which had been exposed to light for 7 days. These showed a total germination of 19.5 percent at the end of the fourteenth day. All seeds were allowed to remain in the dark until the end of the thirtieth day, but no further germination occurred.

In the third series, six lots of seeds were placed on moist filter paper in the dark box. Each successive lot was then exposed to sunlight for one day as follows: Lot 1 was exposed to sunlight the second day, and then returned to the dark box; lot 2 was exposed on the third day and returned to the dark box; lot 3 was exposed on the fourth day, and so on, this procedure being followed until all six lots had been exposed to the sunlight for one day each. Six lots of seeds similarly treated were exposed to the sun-

light for two-day periods; and another series of six lots for three-day periods. No germination occurred in any of the seeds exposed to sunlight for periods of three days or less.

Effect of After-ripening, at Low Temperatures, on Light Sensitiveness

The importance of after-ripening as a prerequisite to germination in many seeds is well established, and it has been maintained by several investigators that the peculiarities of light-sensitiveness exhibited by certain seeds may likewise be influenced by after-ripening. Kuhn (1916), for example, found that while immature seeds of the Amarantaceae are light sensitive, the fully ripened seeds will germinate only in the dark; while Niethammer (1925) makes the general statement that the sensitivity of seeds to light decreases with increase in age. Gardner (1921), on the other hand, states that the seeds of *Verbascum thapsus* L. retain this peculiarity as long as they are viable.

With a view to determining the possible effects of after-ripening on the light-sensitiveness of *Mimulus ringens* seeds, two sets of experiments were carried out. In the first a considerable quantity of seed was placed on moist filter paper, in petri dishes, about two weeks after collection (Oct. 9) and kept in an ice-box at a temperature of 4° C. for four and a half months (until Feb. 28). These seeds were inspected from time to time and distilled water added, as needed, to maintain proper moisture conditions. At the end of this period several lots of 200 each of the after-ripened seeds were sown in petri dishes in the usual manner, and then exposed to three different environmental conditions, with results as follows: (1) cultures placed in bright sunlight in the greenhouse germinated 97 percent in one week; (2) cultures placed in the dark box failed to germinate in six weeks, as was also the case with (3) cultures exposed to "dark oven" conditions. Both of the latter sets of dishes showed germination of 96 percent or more, however, when transferred to bright sunlight at the end of six weeks exposure to darkness.

In the second set of experiments two series of seeds were sown on moist filter paper in petri dishes. One series was placed outside a north window and the other series outside a south window for a period of two and a half months during the winter (December 4–February 28). The seeds in both sets were exposed to temperatures which were almost continuously below freezing with the difference that the first set, being in the north window, was protected from direct sunlight to which the second set, in the south window, was exposed almost daily. Cultures from both sets, when transferred to bright sunlight in the greenhouse on February 28, germinated 95–99 percent within eleven days; but the two behaved somewhat differently when exposed to dark box and dark oven conditions. Seeds from the north-facing exposure showed no germination in either dark box or dark oven,

within a period of six weeks. Seeds from the south-facing exposure, on the other hand, gave 4 percent germination in four days when placed in the dark box, and 2 percent germination in the same period when placed in the dark oven. All seeds in these last two series germinated in excess of 91 percent when transferred to sunlight at the end of the six-weeks period.

Without further experiment it is impossible to assign a definite significance to the differential behavior just described; but it may be inferred that exposure to direct sunlight, under temperature conditions otherwise unfavorable to germination, probably produces some effect which in some way and in some degree acts subsequently to overcome the inhibiting influence of darkness on seed germination. That the observed behavior of the south-exposed cultures is not to be ascribed to more favorable temperature conditions would seem evident from the fact that inspection of these cultures during their outdoor exposure invariably showed the filter paper to be more or less coated with ice crystals.

Effect of Alternating Temperatures on Germination

Liebenberg (1884) and Haack (1912) found that germination in certain light-sensitive seeds could be markedly stimulated by exposure to alternating high and low temperatures. In the author's experiments, cultures of *Mimulus* seed, kept in the dark, were exposed alternately each day to temperatures of 4° C. (for 8 hours) and 25° C. (for 16 hours), for a period of more than two months. The net result was that only one seed out of 1200 germinated. Parenthetically it is of interest to note that this was the only *Mimulus* seed, out of more than 45,000 tested during the course of all the experiments, which germinated in the dark without first being exposed to light of some quality or intensity. All of the above seeds germinated in excess of 90 percent when finally exposed to sunlight.

Effect of High Temperatures upon Germination

Jönsson (1893) was able to induce various light-sensitive seeds to germinate in the dark by exposing them to relatively high temperatures, and similar results have been achieved by Lehmann (1909, 1911, 1912) and Ottenwälder (1914). Bates (1917) states that "For germination either in greenhouse, nursery, or field . . . it is an established fact that light is not required. Light itself cannot affect the seed or seedling which is covered by soil or litter and can only act through the agency of soil temperature."

In the case of *Mimulus ringens*, attempts to induce germination in the dark by raising the temperature have proved unsuccessful. It is possible by this treatment, however, to induce increased germination in diffused light. For example, two lots of seed started in the diffused light box on October 9th, showed 46.5 and 38.5 percent germination respectively on October 31st, when germination ceased. On November 10th, the lot which had germinated 38.5 percent was transferred to the diffused light oven,

where the total germination increased to 95.5 percent within four days. Two other lots of seed were started in the diffused light box and diffused light oven respectively on February 20th. On March 17th, the former lot showed 26.8 percent germination, the latter 90 percent. All ungerminated seeds in both series, when transferred to bright sunlight on April 12th, gave a total germination in excess of 90 percent. In the germination of *Mimulus ringens* seed it seems clear, therefore, that the inhibiting effect of insufficient light can be offset by favorable temperature conditions, but only within certain relatively narrow limits.

Effect of Various Other Conditions on Germination

Krause (1901), Kinzel (1909) and others have listed many non-aquatic plants whose seeds are capable of germinating under water, and it is worthy of note that *Mimulus ringens* belongs in this category. On a basis of several experiments, it can be stated that the germination behavior of *Mimulus* seeds, when submerged in water, differs but little from that of seeds sown on filter paper. In one case, for example, within a period of one week submerged seeds gave a germination of 99 percent in sunlight, 2 percent in the diffused light box, and 48.5 percent in the diffused light oven, these last two figures being raised to 34 and 98.5 percent, respectively, at the end of six weeks. In no case, however, was submergence effective in causing germination to take place in the absence of light.

Hard or impermeable seed coats are recognized as a cause of delayed germination in many seeds, and it has been found by Kinzel (1907–1909), and Lehmann (1909) that germination in the dark can be induced in certain light-sensitive seeds by puncturing the seed coats. Seeds of *Mimulus ringens* so treated showed no reaction in darkness, although the seeds germinated 80 percent when transferred to sunlight after six weeks in the dark oven.

The use of certain mercuric and other compounds has been reported as variously stimulating seed germination. For purposes of experiment three lots of seed were treated for 12 minutes each with a quarter percent solution of commercial "semesan." In one of these lots the seed coats were first broken; in a second the seeds were first soaked in water for one month; in a third the seeds received no preliminary treatment. Considered in order, they showed germination of 58, 64.2, and 64.8 percent in bright sunlight, but no germination in either the dark box or the dark oven.

Gardner (1921) found that germination in darkness, in the case of certain seeds, was somewhat increased by treatment with hot water. Treatment for as short a time as 15 and 30 seconds with distilled water at 75° and 90° C., however, proved fatal in the case of *Mimulus ringens*.

Seeds sown on sand, when placed in sunlight, diffused light box, diffused light oven, and dark oven, gave results essentially identical with those for seeds sown on filter paper.

SUMMARY

1. Freshly collected seeds of *Mimulus ringens* are light sensitive and have practically a 100 percent germination capacity, or viability. Seeds which have been kept in dry storage for many months still retain their light sensitiveness and high viability.

2. Absence of light does not impair the germination capacity of the seeds. Seeds which had been kept in the "dark box" for 6 months showed from 99.2 to 100 percent germination when transferred to bright sunlight.

3. At low light intensities, the germination of *Mimulus ringens* seed is roughly proportional to the intensity of light to which the seeds are exposed, e.g. 75.5 percent in 6.0 foot candle, 34.5 percent in 3.5 foot candle, and 11.5 percent in 1.5 foot candle light intensity. No germination was secured at a light intensity lower than 1.5 foot candles.

4. Brief exposures to light do not stimulate the seeds to germinate in darkness. Seeds given daily exposures to sunlight of one hour or less, for a period of two months, did not germinate. With daily exposures of two hours, germination is slow and incomplete, becoming both more rapid and more complete with increased exposure.

Seeds exposed to sunlight for three days or less failed to germinate and seeds exposed for four days gave very low germination, while seeds exposed for progressively longer periods showed correspondingly greater percentages of germination.

5. After-ripening at low temperatures, both in the dark at 4° C. and out-of-doors in a north-facing exposure, was insufficient to stimulate germination in darkness. Seeds after-ripened out-of-doors at low temperatures, but exposed to sunlight, gave 4 percent germination in four days when transferred to the dark box and 2 percent in the dark oven.

6. Light can be replaced by temperature within certain limits, but increased temperatures alone do not induce germination in darkness. Seeds in diffused light gave somewhat greater germination when the temperature was increased to about 32° C.

7. Seeds submerged in water showed greater germination in the "diffused light box" and "diffused light oven" than seeds sown on filter paper. In no case, however, was submergence effective in causing germination to take place in the absence of light.

8. Rupture of the seed coat did not induce seeds to germinate in darkness.

9. Treatment of seeds with ¼ percent solution of semesan for 12 minutes reduced the percentage of germination and failed to induce germination in darkness.

10. Seeds treated with hot water, at 75° and 90° C. for 15 to 30 seconds, were killed.

11. No significant difference was found in the germination of seeds placed on sand and those placed on filter paper.

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A CYTOLOGICAL AND A GENETICAL STUDY OF *PETUNIA* IV. POLLEN GRAINS AND THE METHOD OF STUDYING THEM [1]

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There is a growing tendency among taxonomists to consider pollen grains of diagnostic value. The significance of the morphology of pollen grains in reference to hybridity and to polyploidy has also been emphasized by students of plants especially during the past ten or more years. As recorded by the senior author (1924) the *n* and *2n* number of chromosomes for the ordinary bedding petunias is 7 and 14 and to this was added, in 1928, the statement that *Petunia axillaris* (Lam.) B.S.P. (*P. nyctaginiflora* Juss.) is characterized by 7(*n*) and 14(*2n*) chromosomes. We would now add to this list the true-breeding species *Petunia violacea* Lindl., and *P. alba* Hort. *Petunia parviflora* Juss. is the smallest flowered petunia yet described but has, contrary to our expectations, 9 and 18 chromosomes. These counts are based on division stages in microsporogenesis and in root tips. Skalińska and Cuchtman (1927), Matsuda (1927), and Malinowski (1928) have recorded 7(*n*) and 14(*2n*) chromosomes for *P. violacea*, but as shown in a recent paper (Ferguson and Ottley, 1932) their studies were unquestionably based on unstable garden forms rather than on the true species.

Pollen grains of three natural species, of one horticultural species, and of many cultivated strains, including polyploids and species hybrids, have been studied in large numbers and under varying conditions; but only those of *P. axillaris* (P14), *P. violacea* (P9), *P. parviflora* (P7), and two tetraploids of distinctly different origin will be discussed in this paper. Pollen grains in relation to chromosome number and to heterozygosity will be considered in a later paper. The large-flowered tetraploid was selected from a family of *4n* plants grown at Wellesley and known in our cultures as population one (P1); while the small-flowered tetraploid was derived from seed collected in California. This plant is a representative of our population four (P4).

MOUNTING MEDIA

The typical pollen grains of *Petunia* are bilateral in origin. If observed in dry air, they are elliptical or lens-shaped in outline when the longer axis is perpendicular to the line of vision, that is, when the grains lie horizontally on the slide. Normally there are three "germ pores," each located on a furrow which extends the entire length of the grain. These furrows are equally distant one from the other and give the grains a somewhat triangular

[1] Published at the expense of the author, out of the order determined by the date of receipt of the manuscript.

shape in end view. In polyploid plants of our cultures the pollen grains are as a rule very irregular in shape and may have four, five, or more germ pores each.

So far as we have been able to determine all studies of pollen grains from the standpoint of the geneticist, and largely from that of the taxonomist, have been based on their appearance in an aqueous medium. When a medium containing even a trace of water is added to a dry mount of the typical pollen grains of *Petunia*, the transition from elliptical to circular outline is so rapid that one watching very closely can not follow the change. As the aqueous medium advances across the slide the grains become instantly circular or, under certain conditions, more or less triangular in form.

Grains were first studied and measured in the various media used by other writers [2]: water, sugar solutions of different degrees of density, fuchsin, lactic acid, alcohol-water-glycerine combination, aqueous and alcoholic solutions of iodine, 10% glycerine, 45% acetic acid slightly colored with iodine, light green, and aceto-carmin. The results with these several media were in general the same; but in all of them one is studying and comparing *transformed* pollen grains, not the true, normally shaped grains. Moreover, it gradually became clear that in certain of these media, perhaps in all, there is not only more or less swelling, depending in part on the medium; but in different mounts made from the same plant, or even from the same flower, the degree of swelling is not always the same. What became still more disturbing was the fact that all grains in a given mount are not affected to the same degree by the aqueous medium, some grains swelling more and others less, some becoming circular, others triangular, and still others irregular in outline. This is especially significant in the species where the grains are normally very uniform in shape. Again, if one watches very closely immediately after a mount is made, in some cases grains here and there may be seen emitting at one point, in the form of a fine spray, a portion of their contents. The ejected material is dispersed throughout the mounting medium and thereafter these partially emptied grains appear normal but they have undoubtedly contracted somewhat with the partial loss of contents.

It thus became desirable, if the study of pollen grains of *Petunia* was to be of any precise value, to secure some medium which would preserve both the normal size and shape of the grains and give clearness of definition. In an attempt to find such a mounting medium, several substances were tried such as very concentrated glycerine jelly, heavy sodium silicate, etc., only to be registered as failures. Finally oily media were tested. Kerosene and nujol apparently preserved the grains without change or distortion in any way. At last a drop of xylol was placed on a dry mount and no change in the grains was apparent. If xylol why not a drop of Canada balsam in

[2] Jeffrey, '14; East, '16; Blakeslee, '21; Sax, '22; Blakeslee and Cartledge, '26; Brink, '27; Matsuda, '27; Melburn and Thompson, '27; Wodehouse, '28; Sinotô, '29; Renner, '29; Dermen ,'30, and many others.

xylol? This, too, was satisfactory. Enough balsam was then added to make the solution sufficiently heavy for permanent mounts. This more concentrated solution also preserved the grains in their normal dry-air form and size. In order to secure the best definition possible for purposes of photographing, a small amount of Bismarck brown was added; but this stain is not essential as the grains stand out with great clearnes in the uncolored balsam. As finally used for the records herein reported the solution was made up according to the formula previously published by the senior author (1932) with a slight change in the proportions used. It consists of one part very heavy balsam to four parts xylol. To this is added a few drops of a supersaturated solution of Bismarck brown in absolute alcohol. In at least one preparation for each strain studied, the pollen was scattered on the slide as evenly as possible, the cover glass placed lightly over the grains, and balsam carefully applied to its edges. This leaves the pollen grains at the center in air while those at the periphery of the mount are in the balsam surrounding the central air bubble. One can thus study side by side pollen grains from the same anther in air and in balsam. Pollen grains may be mounted in heavy balsam with impunity, but for studies in comparative size and form it is not advisable since in the heavier balsam a considerable percentage of the grains do not lie with their longer axes parallel to the slide but often assume an oblique or even vertical position. In studying pollen grains that are tetrahedral or normally more or less circular in outline there would naturally be less disadvantage in mounting them in an aqueous medium than when working with oblong or ellipsoidal grains. But, because of the lack of uniformity of the action of an aqueous medium on the various grains of different mounts or of a given mount, in no case can the results be as satisfactory as when an oily medium is used.

The method of making pollen-grain mounts in balsam was worked out as described above and many preparations studied before Pope's (1925) paper was read. He believes that the most valuable studies of pollen grains can be made only when mounted in dry air. He mounted pollen grains in balsam and says there was "no shrinkage in the grains." But, he adds, "perhaps the most common artefact is the swelling of ellipsoidal, pyramidal and polyhedral grains in balsam producing in such cases a spherical shape." He concludes that balsam mounts are therefore "worthless for making correct determinations." As a result of our own studies covering more than three years and several hundred mounts, we are convinced that no ellipsoidal or elongated pollen grains ever become spherical in balsam if all moisture is excluded prior to and at the time of making the mounts. We shall refer to this again later.

Measurements

In General

A filar micrometer was used for all measurements. To eliminate, in so far as possible, errors due to such slight inaccuracies as may be present in the scale of a most carefully selected micrometer each record was made with one end of the grain, when mounted in balsam, or one side, if measured in an aqueous medium, at the zero line of the scale. The final movement of the scale when making records was always in the same direction, that is, from right to left. If necessary to move in the opposite direction in order to bring a grain into position, the zero line of the scale was carried beyond the grain and then brought back to it by the right-to-left movement. This greatly increases the time involved in making a large number of measurements, but enhances their accuracy. In general it did not seem practical to record measurements for less than one-tenth of a space. With the combination of lenses used, one-tenth of a space on the scale is equivalent to .45 μ. It was our intention to measure only those grains which lay parallel to the slide and free from all other grains at the ends or sides from which measurements were taken. In balsam mounts of ellipsoidal pollen grains, it may not always be clearly evident when a grain is lying slightly oblique to the line of vision rather than exactly perpendicular to it. If slightly inclined to the plane of the slide, a grain would measure somewhat shorter than its true length. The danger then is, even with the greatest care, that some grains may be recorded as too short, never as too long unless carelessly measured.

During the more than eight years that we have grown *Petunia axillaris* (P14) it has remained always true to type, whether continued by selfings or by cuttings. For this reason and because it is a very strong species that flowers and fruits abundantly, we have taken it as our standard for pollen grain studies. If we wish to study the pollen grains of other strains of petunias or of other genera of plants, we first examine the pollen grains of P14. If when mounted in balsam they have the characteristic form for that species under dry air conditions, we then proceed with our study of the pollen grains of the other forms. But if the grains of *P. axillaris* give the slightest suggestion of having broadened out, though perhaps far from assuming a spherical shape, further study is postponed until such a time as P14 gives evidence that atmospheric conditions are correct for securing pollen grains of typical form. It is imperative that one should have some such standard when studying pollen grains of hybrids and polyploids or of any species the form of whose pollen grains has not been previously determined.

Preliminary measurements[3] of pollen grains of various ages were made from material mounted in air, in balsam, and in aceto-carmin. Grains taken

[3] These were made by the junior author, but all measurements herein recorded were made by the senior author.

just before the anthers dehisced, at varying times between dehiscence and the fading of the flower, and from herbarium specimens which had been preserved under dry air conditions for more than a year gave practically uniform results. The only exception to this was found in the fact that there is apparently a somewhat increased tendency to swelling in an aqueous medium when the grains are taken prior to the opening of the anthers. In one herbarium specimen the grains were circular in outline, and in another many grains had germinated, frequently displaying long pollen tubes. In such cases the specimens had evidently been collected directly after a rain or a heavy dew. It is doubtless rarely, if ever, safe to draw conclusions regarding pollen grains from herbarium specimens only, as the taxonomists have so frequently done. It was our intention that all measurements recorded in this paper should be based on pollen grains mounted from two to ten hours following dehiscence. In a few cases, however, they were slightly older, but were never taken more than twenty-four hours after the anthers had opened. When placed in an aqueous medium they were measured immediately after mounting but no haste is necessary when mounted in balsam as the grains undergo no demonstrable change in this medium. For each strain studied mounts of pollen grains were made in both balsam and aceto-carmin from at least three different plants, and at various times during more than two years. One hundred measurements, never more, are recorded from a given mount.

In Balsam

The ellipsoidal pollen grains of the three species of *Petunia* under consideration have normally a maximum diameter slightly more than twice the length of the minor diameter, and taper in line with the longer axis to the more or less obtuse extremities or poles. Matsuda (1927) also reports that the pollen grains of *Petunia* are ellipsoidal in fresh material. In our studies it very early became apparent that in "fresh material" of diploid petunias the form of the grains varies from ellipsoidal or lens-shaped to bluntly tetrahedral to spherical depending on atmospheric conditions. In all the strains of *Petunia* and of other genera studied the grains, with the exception of the abortive ones, are, as already indicated, more or less spherical during rain, after heavy dews or when the air is heavily charged with vapor. The size of the pollen grains of *Petunia* in dry air or in balsam is variable within certain limits for each of the three species, the grains being small, medium, or large corresponding to the general morphology of the species (Pl. XLV, figs. 1, 2, 6, 7, 8, 9). For a comparison of the gross morphological characters of *P. parviflora*, *P. violacea*, and *P. axillaris* consult plates of the earlier paper (Ferguson and Ottley, 1932).

From a consideration of table 1 it will be seen that there is a minimum, a characteristic, and a maximum length of grain for each species. By the expression "characteristic length," we refer to the length shared by 25

TABLE 1. *Lengths of Pollen Grains of Petunia Measured in Balsam. 500 Grains for Each Species. The Width of One Space on the Micrometer is 4.5 Microns. P7, Petunia parviflora Juss. P9, P. violacea Lindl. P14, P. axillaris (Lam.) B.S.P.*

Populations	Spaces on the Filar Micrometer																
	6.8	*6.9*	*7*	*7.1*	*7.2*	*7.3*	*7.4*	*7.5*	*7.6*	*7.7*	*7.8*	*7.9*	*8*	*8.1*	*8.2*	*8.3*	*8.4*
P7	1	2	12	8	10	12	20	30	19	45	57	66	*127*	37	25	14	9
P9																	
P14																	

	8.5	*8.6*	*8.7*	*8.8*	*8.9*	*9*	*9.1*	*9.2*	*9.3*	*9.4*	*9.5*	*9.6*	*9.7*	*9.8*	*9.9*	*10*	*10.1*
P7	6																
P9	1	1	0	2	2	9	4	6	10	11	32	26	37	34	47	*121*	40
P14																	

	10.2	*10.3*	*10.4*	*10.5*	*10.6*	*10.7*	*10.8*	*10.9*	*11*	*11.1*	*11.2*	*11.3*	*11.4*	*11.5*	*11.6*	*11.7*	*11.8*
P7																	
P9	31	15	18	19	12	11	5	2	4								
P14					1	1	0	0	6	3	2	4	8	5	6	10	14

	11.9	*12*	*12.1*	*12.2*	*12.3*	*12.4*	*12.5*	*12.6*	*12.7*	*12.8*	*12.9*	*13*	*13.1*	*13.2*	*13.3*	*13.4*	*13.5*
P7																	
P9																	
P14	23	*125*	35	35	31	37	34	26	27	29	18	13	5	1	0	1	

percent, more or less, of the 500 grains recorded for each species. The remaining 75 percent, more or less, of the grains are scattered irregularly between the two extremes. The record for each species as given in table 1 is compiled from five measurements, of one hundred grains each, based on five different mounts made at different times of the year and from three or more different plants. These sets of one hundred measurements each give, within certain limits, very variable results. For instance the extremes for P14 were registered in but a single count. The minimum length was recorded in the first 100 and the maximum in the third. The limits for both the fourth and fifth hundreds fell at spaces 11 and 13, respectively.

The results, expressed in microns, of 500 measurements for each of the three species recorded in table 1 and in text figure 1 are as follows:

	Minimum Length	Maximum Length	Average Length	Length at Mode
P7	6.8 spaces or 30.6 μ	8.5 spaces or 38.25 μ	35.2296 μ	8 spaces or 36 μ
P9	8.5 " " 38.25 μ	11 " " 49.5 μ	44.7174 μ	10 " " 45 μ
P14	10.6 " " 47.7 μ	13.4 " " 60.3 μ	55.017 μ	12 " " 54 μ

A more graphic presentation of the comparative lengths of the pollen grains in these species is given in text figure 1. It will be noted that the average length in the three species may be slightly *over* or slightly *under* the length

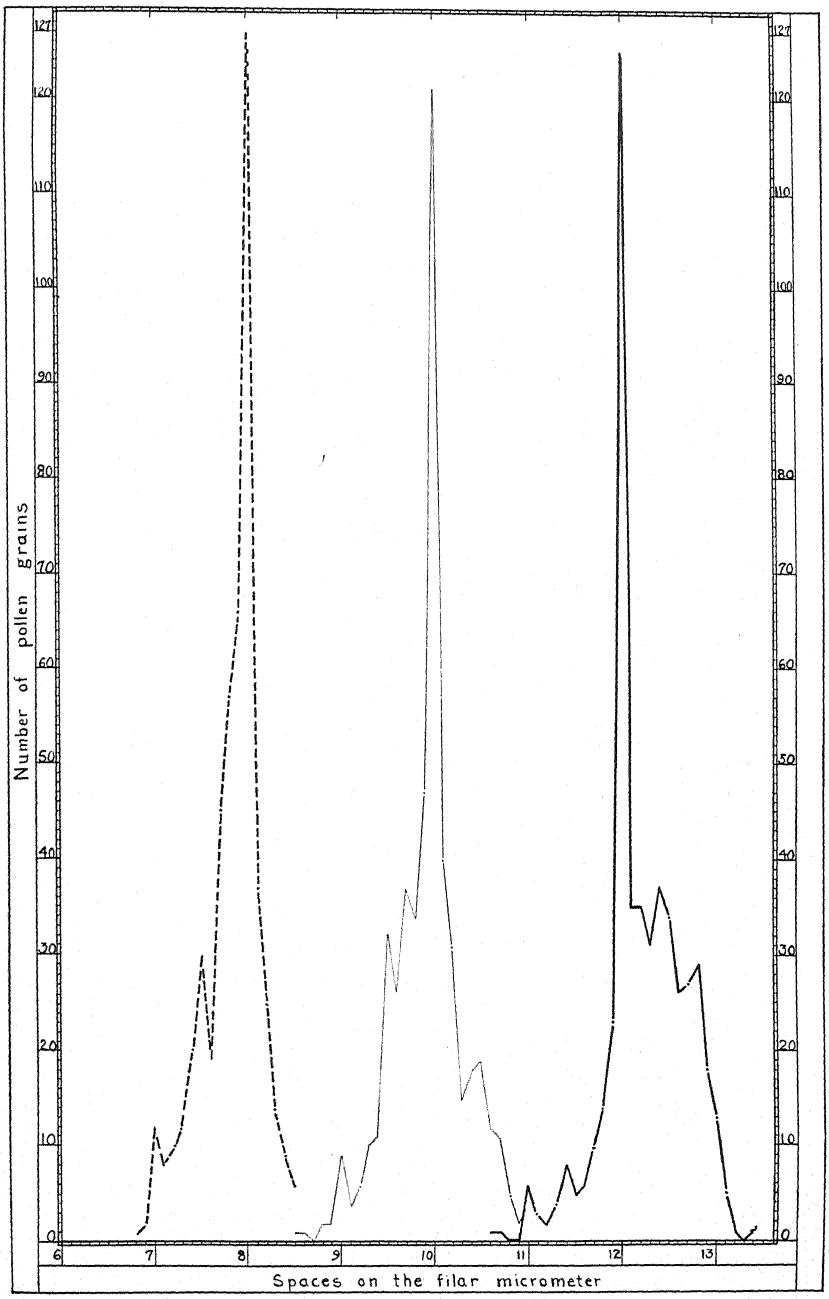
at the mode. It has seemed to us that the mere statement of averages alone is of little value in such a study. The extremes may be of such unusual size for a species as to affect the average very decidedly and, therefore, the average gives no true picture of the typical pollen grain. The differences in the characteristic lengths of the pollen grains, as indicated by the modes in text figure 1, in passing from the species with the shortest to that with the longest grains, is in each case 2 spaces or 9 microns.

The anthers of *P. parviflora* are small and contain comparatively few pollen grains. It often requires the pollen of several flowers for a single satisfactory mount. But the pollen is abundant in *P. violacea* and *P. axillaris* and for both of these more than a thousand grains in addition to those recorded in table 1 were measured. The results of these additional measurements were in complete accord with the records given in the table. They were not incorporated in the table not only because of the difficulty of securing such a large number of measurements for P7, but because the larger number would greatly increase the length of text figure 1 and would give no additional information. It is this constancy of size within certain limits together with the uniformity of shape and of the character of exine that led Miss Ottley and the senior writer (1932) to ascribe diagnostic value to the pollen grains of these three species of *Petunia*.

In Air

Because of the tendency of pollen grains of *Petunia* to clump together in air and because of the differences of the refractive indices of balsam and of air, the grains may be much more easily and critically studied and measured in the former medium. Due to the difficulty of getting each of several grains sharply focused when photographing dry pollen grains mounted in air, they appear somewhat more obtuse at the poles than when photographed in balsam. (Compare figures 1, 6, 8 with figures 2, 7, 9, Plate XLV.) But as a result of repeated measurements, when grain after grain is brought into careful focus on the scale of the micrometer, we have been able to demonstrate very clearly that graphs, for the length of grains for a given species, are practically identical when the measurements are based on two mounts, one in air and the other in balsam. At least they as nearly conform as do the records based on two different mounts in balsam. This conclusion is based on the measurement in air of more than 1000 grains about equally divided among the three species.

The pollen grains of tetraploid petunias adhere in little clusters in air and are too variable in shape to permit records based on measurements either in air or in balsam to be of any value. A variable amount of ellipsoidal grains may be scattered among the various forms, but they are relatively so few in number that a record of their lengths could be of no significance in a comparative study of pollen grains. The other grains assume almost every possible form. They may be lens-shaped with a slight dis-



TEXT FIG. 1. Graphs showing comparative lengths of 500 pollen grains in each of 3 species of *Petunia*. Pollen grains mounted in balsam. Each space on the micrometer is 4.5 microns. ——— = *P. axillaris* (Lam.) B.S.P. ——— = *P. violacea* Lindl. ------- = *P. parviflora* Juss.

tortion on one side; they may be almost circular in outline with a rim suggesting a plate; or rectangular with cross markings suggesting an envelope; or tetrahedral; or elongated cones in outline. Frequently they suggest a half melon with one lengthwise slice removed (Pl. XLV, figs. 10, 12). Naturally only a few of the variations in form can be shown in a single photograph including one percent, more or less, of the grains in a given mount. Such variations in form are undoubtedly due, as suggested by Hoar (1927), Woodworth (1929), Matsuda (1930) and others, to irregularities in microsporogenesis. We shall not discuss them further here as they are to be considered in a later paper.

In Aceto-carmin

We have spoken earlier of the tendency of pollen grains, normally uniform in shape, to vary in the degree of swelling, to assume various forms, and even to emit a portion of their contents when mounted in an aqueous medium. A careful study of the conditions leads one to the conclusion that these irregularities are due to variations resident in the grains themselves. Undoubtedly the grains in a given anther have not all reached the same degree of maturity at the time of dehiscence, especially as regards the thickness and the degree of cutinization of their walls. If the wall of a grain is only very slightly thinner or less cutinized than that of others it is correspondingly more elastic and would consequently permit of greater extension in the presence of water than would the thicker more heavily cutinized walls. This is especially pertinent to the pollen grains of polyploids and of certain cultivated diploids. In these the mature grains not only vary greatly in shape and size but in the thickness of their walls as well. The smaller grains often have proportionately thick walls in comparison with the amount of their cytoplasmic content and may undergo no appreciable swelling.

In his study of the pollen grains of *Linaria genistifolia* Mill. and other plants, Piech (1922) finds variations in form and in the thickness of the walls, and concludes that they are due in large measure to variations in external factors, especially of temperature and moisture. Kawecka (1926) also attributes the variations in pollen grains of *Oenothera* to environmental factors, in particular to cold. Temperature undoubtedly influences meiosis and indirectly the size and shape of the pollen grains. But it is probably equally true that the presence or absence of water during the growth and maturation of the pollen grains has a marked influence both on the walls and on the protoplasmic contents of the mature grains.

The irregularity of behavior manifested by pollen grains in an aqueous medium may also be due, in part, to the degree of desiccation which the cytoplasm of the individual grains has undergone at the time the mount is made. It is not only possible but probable that pollen grains at the time most favorable for their dispersal contain no free water but only what

Lepeschkin (1930) calls "organic liquid." It is clearly evident from their behavior in balsam that their walls are dry at the time of shedding. Furthermore, they may absorb sufficient moisture through the germ pores to cause them to assume a spherical form and the exine remain unwetted. The pollen grains of P14 shown in figure 3, Plate XLV, were collected in the morning after a very heavy dew and mounted immediately in balsam. They did not swell in the balsam, as Pope (1925) asserts in his studies of pollen grains, for they were all spherical when placed on the slide, as revealed by an examination of them under the microscope before the balsam was applied. Furthermore grains from other anthers of the same flower and from other flowers collected at the same time and mounted at once in air were spherical in form. These were allowed to remain uncovered on the slide in the dry air of the laboratory, and when examined some hours later they had assumed their normal ellipsoidal form. But when the absorption of moisture passes a certain critical point, the grains when mounted in balsam are each surrounded by delicate films of water (Pl. XLV, fig. 4). It will be noted that the grains shown in figure 4 are not only surrounded by films of moisture but are somewhat larger than are those shown in figure 3, indicating that more water has been absorbed in the former case with resultant increase in size.

When viewed in aceto-carmin or other aqueous media the normal pollen grains for species of *Petunia* are ordinarily circular in outline but they may sometimes present a slightly elongated, bluntly triangular figure. Matsuda (1927) finds that this variation of form in the presence of water is associated with the age of the anthers, the spherical form being characteristic of young anthers and the tetrahedral of older ones. We have not been able to trace such a connection and it has seemed to us more probable that it is due to the thickness of the walls and more especially to the particular phase of the protoplasm in the different grains at the instant that the water is applied. The grains of polyploids in aqueous media may resemble very closely, throughout the whole preparation, the pollen grains of true breeding diploids in similar media. At other times the grains of the same polyploids or of other strains of polyploids present a very motley appearance being circular, square if four pores are present, bluntly elliptical, swollen at one pole, or irregular in outline (Pl. XLV, figs. 5, 11, 13).

Table 2 gives a comparison of the pollen grains of three species and two tetraploids based on the length of the longest diameter of the grains as measured in aceto-carmin. The same records are presented in the form of graphs in text figure 2. It has been shown earlier that pollen grains of *Petunia* have the same form and size in balsam and in dry air. If one compares the record given in table 1 with that given in table 2 it is at once apparent that the measurements in balsam and in aceto-carmin practically agree as to the relative sizes of the pollen grains in the three species, and table 2 further shows the greater range in size of the grains of the tetraploids over

TABLE 2. *Comparison of Pollen Grains of Petunia, Mounted in Aceto-carmin, Based on the Length of the Longest Diameter of 500 Grains for Each Population. One Space on the Micrometer Equals 4.5 microns. P7, Petunia parviflora; P9, P. violacea; P14, P. axillaris; P1, Large-flowered Tetraploid—2987.3; P4, Small-flowered Tetraploid —30126.77.*

Populations	Spaces on the filar micrometer															
	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6	6.1	6.2	6.3	6.4	6.5	6.6
P7	1	6	8	20	16	28	40	54	76	90	58	48	32	12	6	3
P9																
P14																
P1																
P4											2	1	2	2	1	2

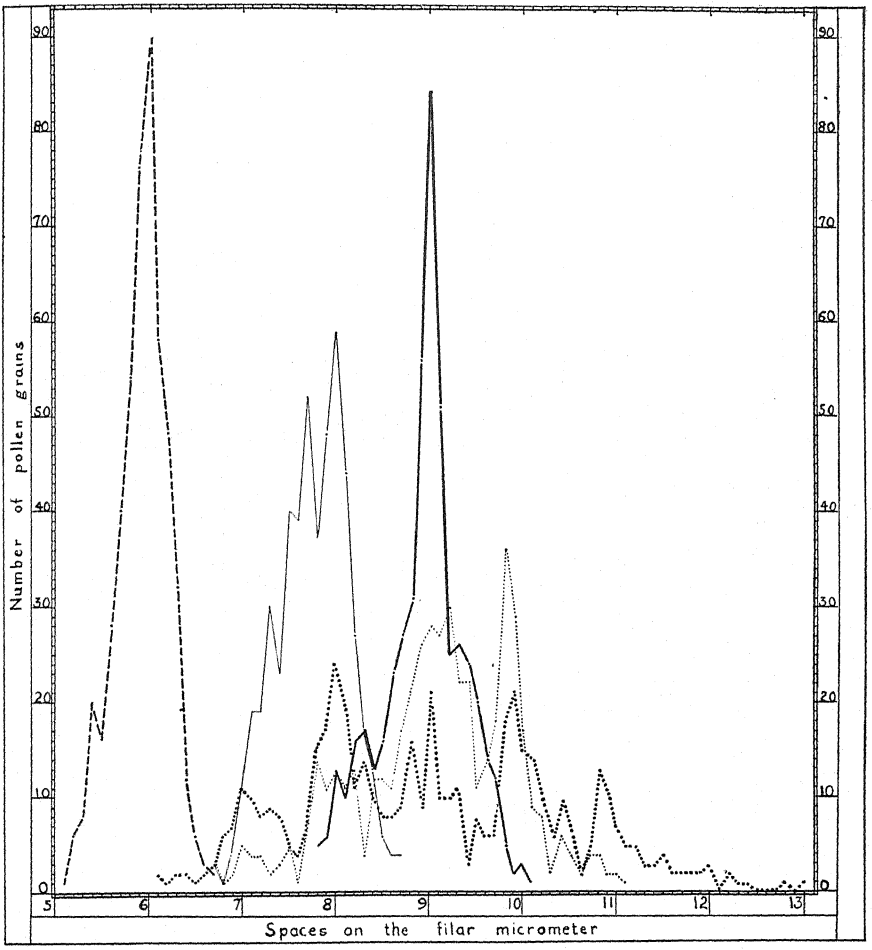
	6.7	6.8	6.9	7	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8	8.1	8.2
P7	2															
P9	3	1	4	11	19	19	30	23	40	39	52	37	48	59	44	27
P14												5	6	13	10	16
P1	3	1	2	5	4	4	2	3	5	1	7	14	11	13	11	13
P4	3	6	7	11	10	8	9	8	5	4	7	15	17	24	19	11

	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9	9.1	9.2	9.3	9.4	9.5	9.6	9.7	9.8
P7																
P9	17	13	6	4	4											
P14	17	13	16	23	27	31	56	84	51	25	26	24	20	14	12	5
P1	4	12	12	11	17	21	26	28	27	30	22	22	11	14	18	36
P4	14	10	8	8	9	16	9	21	10	10	11	3	8	6	6	18

	9.9	10	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	10.9	11	11.1	11.2	11.3	11.4
P7																
P9																
P14	2	3	1													
P1	29	17	9	8	2	6	4	2	4	4	2	2	1			
P4	21	15	14	10	6	10	7	2	5	13	10	7	5	5	3	3

	11.5	11.6	11.7	11.8	11.9	12	12.1	12.2	12.3	12.4	12.5	12.6	12.7	12.8	12.9	13
P7																
P9																
P14																
P1																
P4	4	2	2	2	2	3	0	2	1	1	0	0	0	1	0	1

those of the species. The grains of P4 well overlap those of P7, cover the whole range of P9 and P14 and extend twenty-nine spaces or 130.5 microns beyond P14. But they do not give any indication of the constancy in form of the grains in the species and the marked irregularity of form in the tetraploids. Furthermore if one has the graphs shown in text figure 1 in mind, it needs only a glance at text figure 2 to be convinced that the measurements in the aqueous medium give a very erroneous picture as regards the relative lengths of the pollen grains in the three species. The results,



TEXT FIG. 2. Graphs showing comparative sizes of 500 pollen grains in each of 3 species and 2 tetraploids of *Petunia*. Grains mounted in acetocarmin. Each space on the micrometer is 4.5 microns. ——— = *P. axillaris*, ——— = *P. violacea*, ------- = *P. parviflora*, ····· = P1, large-flowered tetraploid, ····· = P4, small-flowered tetraploid.

expressed in microns, of 500 measurements for each of five populations as recorded in table 2 and text figure 2 are as follows:

	Minimum Diameter	Maximum Diameter	Average Diameter
P7	5.1 spaces or 22.95 μ	6.7 spaces or 30.15 μ	26.6679 μ
P9	6.7 " " 30.15 μ	8.7 " " 39.15 μ	34.8957 μ
P14	7.8 " " 35.1 μ	10.1 " " 45.45 μ	40.14 μ
P1	6.7 " " 30.15 μ	11.1 " " 49.95 μ	40.7457 μ
P4	6.1 " " 27.45 μ	13 " " 58.5 μ	40.4235 μ

If one notes the fact, that the average diameter for P14 and P4 differ by but .28 of a micron, in connection with the graphs for these two populations

and with figures 5, 11, 13, one must conclude that average diameters of pollen grains in an aqueous medium have little or no significance when comparing pollen grains of diploids and tetraploids, at least in so far as the pollen grains of *Petunia* are concerned.

In his studies of pollen grains, Wodehouse (1928) used as mounting media either an alcohol-water-glycerine combination or Brandt's glycerine jelly. In discussing Mohl's (1835) studies he says: "He clearly understood the importance of observing the grains in the moist condition." He cites Smith (1876) and Pope (1925) as examples of students who "insist that pollen should be observed in the dry form," and says that Bennett's (1875) "figures are mostly lacking in detail because his observations were confined to dry material." From the data presented in the present paper it seems clear that both dry air and balsam as mounting media give a truer picture of the size and form of pollen grains than does any aqueous medium.

Summary

1. The haploid number of chromosomes in the true breeding species *Petunia violacea* Lindl. and *P. alba* Hort. is 7, the somatic number in these species being 14. The *n* number of chromosomes in the small flowered species, *P. parviflora* Juss, is 9, the *2n* number is 18.

2. The pollen grains of *Petunia* are normally ellipsoidal. The two natural species named above and *P. axillaris* have, each, a characteristic length of grain.

3. The value of averages in quantitative studies is questioned.

4. Pollen grains are not appreciably changed in form or in size when mounted in a thin solution of Canada balsam in xylol.

5. Whatever the normal form of pollen grains, they become more or less spherical in an aqueous medium.

6. No aqueous medium, heretofore used, for studying pollen grains was found to act uniformly on all the grains of a given mount.

7. As a result of our observations it is believed that descriptions of pollen grains, as observed in an aqueous medium, are of doubtful value.

8. The pollen grains of polyploid petunias present a mixture of various forms and sizes.

9. While there are such marked differences in the appearance of pollen grains of species and of polyploids in *Petunia*, the two frequently can not be distinguished when mounted in a medium containing water.

10. Since pollen grains are very susceptible to atmospheric conditions, it is essential that a "type form" of grain be observed before mounting for study the grains of any species or of a cultivated strain the form of whose grains is unknown.

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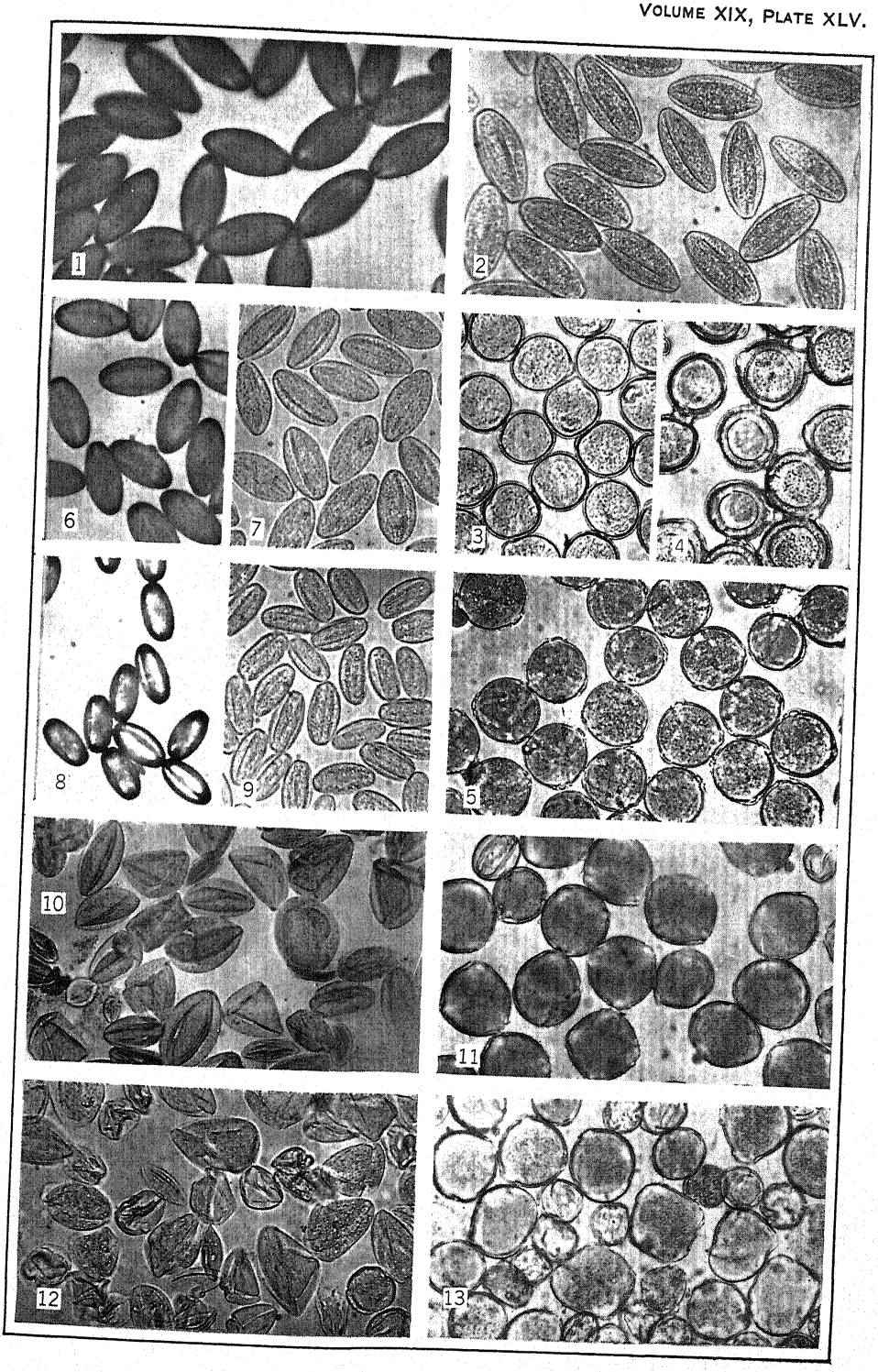
DESCRIPTION OF PLATE XLV

Pollen grains of *Petunia*. The grains were magnified × 370 and reduced by one-third in reproduction.

FIG. 1. *P. axillaris* in air.
FIG. 2. *P. axillaris* in balsam.
FIG. 3. *P. axillaris* after a heavy dew.
FIG. 4. *P. axillaris* saturated with moisture.
FIG. 5. *P. axillaris* in aceto-carmin.
FIG. 6. *P. violacea* in air.
FIG. 7. *P. violacea* in balsam.
FIG. 8. *P. parviflora* in air.
FIG. 9. *P. parviflora* in balsam.
FIG. 10. P1, large-flowered tetraploid, in balsam.
FIG. 11. P1, large-flowered tetraploid, in aceto-carmin.
FIG. 12. P4, small-flowered tetraploid, in balsam.
FIG. 13. P4, small-flowered tetraploid, in aceto-carmin.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THE STRUCTURE OF THE BRYOPHYTE PLASTID WITH REFERENCE TO THE GOLGI APPARATUS

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Do plant cells possess a structure comparable to the Golgi apparatus of animal cells, and, if so, what is it? For some years it has been held by Bensley (1910), Guilliermond (1930), and Parat (1928) that the plant vacuole is homologous to the Golgi apparatus. They maintain that the classical apparatus of Golgi is merely an idea, having no real existence within the animal cell. What is observed is an artifact brought about by the precipitation of osmium and silver upon surfaces within the cell. The realities of cellular structure are the vacuoles and mitochondria. Since, however, the work of Nassonov, Bowen, Gatenby, Morrelle, Beams and many others presents evidence for the conclusion that the Golgi body is cytoplasmic in nature, much doubt is cast upon the concept that the plant vacuole and the Golgi body are homologous structures.

Bowen (1926*c*) at one time put forward the hypothesis that the plastid might be the plant counterpart of the Golgi apparatus. He later abandoned this idea in favor of his osmiophilic platelet hypothesis (Bowen, 1928). This latter concept has, however, not found great acceptance. Platelets have been reported by Patten, Scott, and Gatenby (1928) in the meristematic cells of *Vicia* and *Hyacinthus* roots and in *Pisum* shoots. Kiyohara (1930) describes them in the growing points of *Vicia faba, Hydrilla verticillata,* and *Elodea canadensis* roots and in *Hydrilla verticillata* and in *Elodea canadensis* stems. He believes them to be proplastids. Weier (1931, 1932*a*) has found them in spermatogenous, archesporial, and epidermal cells of *Polytrichum commune.* They are also large and numerous in the young androcytes of *Pellia epiphylla* (unpublished data). In all of these cases it is very noticeable that the tissues examined have been, with the exception of the epidermis, meristematic in nature. It also would appear that the platelets are present only in tissues which do not contain mature chloroplasts. This is not sufficient cause for one to maintain that they are not analogous to the Golgi apparatus, as Bowen claimed, or that they may not be of importance in

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cellular metabolism, but it most certainly does indicate that their importance as universal plant elements should not be stressed quite as strongly as Bowen did, at least not until we know more of their function and life history. That they are not, as Guilliermond maintains (1930), swollen mitochondria seems very evident from an examination of successful preparations.

The present writer in a series of papers has reported a rather striking resemblance as occurring between the plastid and the Golgi apparatus (Weier, 1931, 1932*a*, 1932*b*). This similarity is shown in structure, in behavior during meiosis and spermogenesis, in function, and in many similar reactions to fixation. These previous studies were of necessity confined almost entirely to a comparison of my preparations with the published reports and drawings of other investigators. Recently Professor E. B. Wilson has been kind enough to send me a number of the late Professor Robert Bowen's preparations, and Professor H. W. Beams graciously placed at my disposal a number of his slides. With this material available I have been able to make a careful slide-for-slide comparison of the Golgi apparatus and the plastid.

This comparison falls naturally into two parts. In the first section I have made a slide-for-slide comparison between the Golgi body and the plastid. In doing this I have not taken into consideration the various physiological stages which both plastid and Golgi body may pass through during a secretory cycle. The reason is obvious—no one has yet worked out the cytological aspects of starch elaboration, while the secretory cycle of the Golgi zone is in many cases but imperfectly understood. I have merely chosen a number of stages in which the two forms have a sufficiently striking resemblance to merit attention. There are indications, however, some of which have been brought out in previous reports (Weier 1931, 1932*a*, 1932*b*) and another which will be illustrated in a later connection in this paper, which point to the conclusion that these similar forms are in all probability performing like functions in comparable phenomena. In addition it must be pointed out that in only a very few cases are the comparisons absolutely identical. Again the reasons are obvious—physiological activity undoubtedly determines the structure and form of the Golgi zone and plastid. Just as there must be a chemical difference between starch elaboration and enzym elaboration there will be various differences in the detailed structure of the two forms.

The second part of the description is based on a comparison of my *Anthoceros* preparations and Bowen's (1926*a*, 1926*b*, 1926*c*, 1926*d*) description of the history of the secretory cycle of certain gland cells. In this study I am not attempting to give a history of plastid behavior during a secretory cycle. My preparations, ranging from plastids void of starch to those gorged with it, most certainly suggest that there is as clearly a cycle of starch elaboration within the plastid as there is a secretory cycle in the Golgi apparatus of the gland cell, but since there are many details as yet unexplained I do not wish at this time to go into a description of the cytological aspects of starch elaboration. The fact that plastids at certain stages exist without starch and at others

with varying degrees of starch is sufficient and all that will be considered in this section of the present paper.

Slide-for-slide Comparison of Golgi Body and Plastid

I have already pointed out that the plastid present in the archesporial and androgonal tissue of *Polytrichum commune* is surprisingly similar to the discrete dictyosome form of the Golgi apparatus. In Plate XLVI, figures 1 and 2, this resemblance is most strikingly illustrated. Figure 1 is taken from *Polytrichum,* while figure 2 represents a portion of a nerve cell from the spinal ganglion of a grasshopper. The resemblance is so complete that no further remarks need to be made.

The next few figures (figs. 3 to 8), are examples of the different types of fixation that may be obtained with both structures. The most frequently observed metallic impregnation picture of the archesporial plastid of *Polytrichum commune* is that in which the plastonema is heavily blackened at its interfaces with the cytoplasm and with the plastosome, leaving what is in all probability the major part of the plastonema itself as a perceptibly lighter line which is bordered by two heavily impregnated surfaces (fig. 3). The plastosome is easily discernible, though but slightly differentiated from the remainder of the cytoplasm. In osmic acid impregnations of the Golgi apparatus in the intestine of the rat one frequently meets with a surprisingly similar structure (fig. 4). The apparatus is rather dictyosome-like. The deposition of the osmium is principally confined to the surfaces of the chromophilic portion, leaving a clear unstained line existing between these two blackened ones. There is faintly discernible a slight differentiation of the cytoplasm which is surrounded by the blackened Golgi apparatus.

Less frequently one encounters an impregnation in which the chromophilic substance of both structures no longer forms two clear cut single lines. Instead two rather irregular broken lines border the unblackened interior material (figs. 5 and 6). The plastid (fig. 6) is taken from the archesporial line of *Polytrichum commune* and the Golgi apparatus from the rat intestine. In still other cases the metallic impregnation is reduced to two slender delicate lines bordering the chromophobic inner substance (figs. 7 and 8). Figure 8 represents plastids from the archesporial line of *Polytrichum.* That these different conditions are not wholly due to purely physical differences of intensities of osmium impregnation is clear from the fact that the examples of the Golgi zone were all taken from the same section while in the case of the plastid, figure 8, which represents the least type of blackening, was taken from a sample of material that had been impregnated for nine days at 35° C., the longest time which any of my material remains in the osmium solution.

Whether or not we consider the plastid of the archesporial tissue of *Polytrichum commune* and the Golgi zone in the intestinal cells of the rat to possess exactly similar structure, we must admit that the type of osmium

impregnation is quite identical. In each case a filamentous or plate-like structure so reacts to osmification that the lower oxide is deposited at the interfaces of the chromophilic portion with the cytoplasm proper and with other specialised regions of the Golgi zone or plastid as the case may be. The type of osmification varies from those in which it is quite heavy, forming two intensely black lines bordering a lighter interior substance, through instances in which these black lines are broken, to still other cases where the osmification has been very slight.

My next comparison utilises the Golgi apparatus of the anterior lobe of the rat hypophysis and the picture of the plastid sometimes met with in the somatic cells which form the walls of the young antheridium in *Polytrichum commune*. In each case the osmium is again deposited along the interfaces between the ordinary cellular cytoplasm and the specialised regions forming the Golgi zone or plastid. In addition there are cross lattices which give to this chromophilic portion the appearance of a ladder (figs. 9 and 10). Figure 10 is the plastid. The Golgi zone (fig. 9) may possibly have attached to it a less chromophilic substance which is not present in the somatic plastid.

The somatic plastid of *Polytrichum, Catharinaea, Anthoceros,* and *Pellia* after fixation is most frequently observed as a regular platework in the interstices of which the starch is deposited. So far as I am aware the Golgi structure never presents the regular, sometimes almost geometrically perfect, figure exhibited by the plastid. It does, however, at certain stages in many glands, form an irregular closed net or platework, with the secretory droplets making their first appearance in the openings of this structure. (Bowen 1926*a*, figs. 5, 6, and 34; 1926*c*, figs. 4, 5, 14, 16, 17, 18, and 69.) Figure 11, drawn from one of Professor Bowen's preparations, represents the Golgi apparatus as observed in the pancreas of a cat. The secretory cycle is probably not far advanced because the apparatus is still rather small, and because a closed network lacking granules was observable in the cell. The plastid chosen for comparison is taken from a young spore of *Polytrichum commune* (fig. 12). In spite of the fact that we do not have the closeness of resemblance noticed in the case of the spinal ganglion cell of the rat and the archesporial plastid, I believe the similarity is close enough to warrant serious consideration in any comparison of this sort. It seems that in both cases there is a specialised zone of cytoplasm bounded and traversed by plates of some chromophilic substances. The Golgi apparatus would, as the secretory cycle progresses, become more irregular, finally open up and eventually break into fragments (Bowen 1926*a*, figs. 106, 107, 108). I have not as yet carried my investigation of spore development beyond the stage depicted here, but there is some evidence which suggests that the plastid platework, too, may break down in later stages (Weier, 1931).

The preparations of the paratoid gland of the cat, which were made available to me, beautifully illustrate the platework structure of the Golgi zone and its relationship with the secretory granules (fig. 13). The platework is,

of course, quite irregular and in most cases does not form a completely closed system. The secretory granules may be observed in the interstices of the platework. Furthermore, the platework almost completely fills the cell. The single large plastid of *Anthoceros* makes a most striking comparison with many Golgi bodies of this type. The plastid platework is decidedly more regular and it always forms a closed system. Starch, as shown by polarized light, and represented in figure 14 as dark gray patches, almost completely fills the interstices of the platework.

As the secretory cycle of the paratoid gland cell progresses the Golgi apparatus becomes quite irregular and finally fragments into discrete portions which extend here and there between the secretory granules (Pl. XLVII, fig. 16). I have observed a comparable picture to occur in the plastid of the sporophyte of *Anthoceros* but whether or not it will fit a concept similar to that of the secretory cycle of the Golgi zone I am not yet prepared to say (fig. 15). Its remarkable likeness to the Golgi zone, however, makes it of more than passing interest. It is a plastid which is gorged with starch granules and in which the platework has disintegrated into irregular pieces which extend between some of the granules of starch.

The Golgi apparatus of the epididymis of the cat and rabbit is one of the most diversified of structures which have been described under the heading of the Golgi apparatus. It varies in structure from one end of the gland to the other, as well as during the secretory cycle. I have already made one comparison between the plastid and the secretory region of this gland (Weier, 1932*b*). In this case the description of the Golgi zone was given by Parat (1928). I have searched the preparation of the caput region of the epididymis from Bowen's collection but have been unable to find anything to fit Parat's description. Other sections, however, of other regions of the gland might yield different results. Figure 17 of the Golgi zone in the epididymis cell and figure 18 of the archesporial plastid of *Polytrichum commune* do, however, give an example of the type of similarity found. The resemblance is similar to that shown in figures 5 and 6 and needs no further comment, except that it seems probable that the apparatus in the epididymis cell is formed of a plate or ribbon of somewhat greater width than is the plastonema of the plastid. I was unable to confirm this from the slide at my disposal but some of Bowen's figures clearly show that in a good side view of the cell the Golgi apparatus extends as a plate for some little distance. In the caudal region of the epididymis this plate becomes quite extended and it frequently is vacuolate (Bowen 1926*c*, fig. 45). In this condition it is reminiscent of some non-starch-containing plastids of thallus of *Anthoceros* (fig. 19).

The *Elodea* plastid apparently differs markedly from the plastids of *Zea mays* (Weier, 1932*b*) and the bryophyte plastids mentioned here. It consists of a heavily staining, relatively large rod, cap, or handle to which is attached an oval or dome-shaped non-staining mass. This latter region is shown under the polarizing microscope to be starch (fig. 20).

The Golgi bodies present in the mucous cells of the salivary gland of *Limax* (Bowen, 1926*a*, fig. 65), in certain stages of secretion are characteristically of this same structure. They are perhaps more numerous and somewhat smaller, probably not visible in the living cell and more easily destroyed by fixation, but their structure and probably their function is sufficiently similar to make it necessary to consider them in any comparison between plant and animal cytoplasm.

Secretory Cycle in Golgi Body and Plastid

In describing and comparing the different forms of the plastid and the Golgi body in relation to some sort of a secretory cycle it is necessary to proceed with a good deal of caution. Bowen, himself, was not too certain of the early stages and to the writer's knowledge the description of the uniformly staining mass present at the beginning of the cycle has never been confirmed by other investigators. Nassonov (1923), in his account of the secretory cycle, does not describe any of the earlier stages other than in the form of a network. On the other hand for Morelle (1926) the heavily blackening plates of the classical Golgi body are due to fixation, the actual Golgi structure always being a "spot" of specially differentiated cytoplasm.

Although in the plastid there is a clear and concise range of stages from non-starch-containing plastids to those which are gorged with it, I am hardly yet ready to fit these changes into an elaborate secretory cycle. There may be, for instance, two types of plastids present in *Anthoceros*. Then the meaning of the platework structure which is so clearly absent from the living plastid and which figures in no theory of the structure of the living plastid, but yet which clearly bears some relation to starch elaboration, is a moot question which must be settled before a definite knowledge of a secretory cycle may be ascertained.

In describing the various forms of the Golgi body and the plastid from the point of view of a secretory cycle let us not consider the cycle as a definitely established concept. We shall rather compare plastid and Golgi body when visible secretions are absent, when a few products of elaboration are present, when a somewhat larger number are enclosed within the elaborating structure, and when the secretory regions are gorged with the products of elaboration. Whether or not these stages eventually may be placed in a well defined cycle is a matter for future research to decide. The significant fact for the present study is to discover whether or not the plastid and Golgi body resemble each other when somewhat similar amounts of secretory material are present within the cell.

According to Bowen (1926*a*) there is present in many glands a stage in which certain cells are absolutely devoid of secretory granules. Under these conditions the Golgi apparatus in a number of glands, the mucous cells of the submaxillary of the cat, the white portion of the Harderian glands of the rabbit, the Mebomian gland from the upper eye-lid of the cat, and the

epididymis of the cat and the rabbit, for instance, is present in the form of a small, evenly staining, blackening mass lying between the nucleus and the lumen of the cell.

My *Anthoceros* material was collected on the campus of St. Lawrence University and brought into the laboratory where it was killed some hours later. Whether the fact that it was thus removed from the sunlight for a short time before fixation accounts for the absence of starch in certain plastids I do not know, but the plastids in the most superficial cells of the thallus were devoid of starch when viewed under the polarized light of crossed nicols prisms. With this absence of starch the plastid always fixed evenly and smoothly without the slightest suggestion of a platework figure (figs. 21, 22), thus corresponding closely to Bowen's description of the Golgi body in some types of cells at the commencement of the secretory cycle. Plastids heavily or lightly blackened by the Kolatchev technique (fig. 23), or those fixed according to Parat's modification of Helly and stained either with Kultschitzky's or Heidenhain's haematoxylin show exactly the same lack of structure (fig. 21). At times this starchless plastid would exhibit a rather vacuolate structure (figs. 19 and 23).

In the very earliest stages of secretion the evenly blackening Golgi apparatus opens up slightly so that short loops or bulging plates extend unevenly from the more homogeneous mass. The plastid of a somewhat corresponding stage is marked by numerous and rather large "vacuoles" in which starch appears (fig. 24). Here again the type of fixation, Kolatchev or Parat, does not effect the type of picture obtained.

A comparison between the plastid and the Golgi zone when they contain a large quantity of secretory material has already been made (figs. 13 and 14). The Golgi zone is composed of a rather irregular platework in the interstices of which the secretion first appears (fig. 13). The same thing is essentially true of the plastid, the difference being in the more regular picture presented by the plastid (fig. 14).

When both structures are gorged with secretory material there seems again to be some degree of correspondance in their structure (figs. 15 and 16). The platework has fragmented, and the short pieces ramify in an irregular manner between the starch or digestive enzym, as the case may be.

It may be said that the plastid and the Golgi body are each specialised regions of cytoplasm which elaborate specialised non-living compounds for the use of the organism. After fixation with Golgi techniques their structures correspond in that they exist either as plateworks or as discrete dictyosomes. In addition the pictures exhibited by the two bodies after fixation agree quite well in relation to the amount of secretory material that is present in each cell. In each case the visible end product of the chemical reactions taking place within the corresponding regions apparently bears some relation to the specialised cytoplasm which reduces the osmium tetroxid. Their differences lie in the fact that the plastid is more resistant to certain fixation techniques

and that although the Golgi zone is well preserved by mitochondrial fixatives it is not usually possible to stain it after treatment with them; whereas the plastid is quite easily stainable after fixation with this group of fluids. It is also interesting to point out that both structures pass through a period of fragmentation such that it is possible only with great difficulty to differentiate them from mitochondria.

Discussion

Homology

It must be pointed out that even though there most certainly exists a most striking correspondance between plastid and Golgi body we cannot as yet speak of them as homologous structures. They may be homologous, but it is impossible for us to know, at present, whether or not they ever had a common origin, from an evolutionary point of view. Until future research either gives sufficient evidence to decide this point or shows our inability to decide it, it seems best that our present knowledge be not further confused by the concepts conveyed in the term "homologous." The structures are most certainly comparable but in so far as we know they are not homologous.

The Plant Vacuole and the Golgi Apparatus

It has been pointed out that investigators basing their argument on the similarity of the network structure of the plant vacuole to the Golgi zone are maintaining that there exists no such structure as the Golgi apparatus in the animal cell. For instance, Owens and Bensley (1929) have maintained that in the light of their work "it is obvious that the 'magnificent apparatus of Golgi' as displayed in the osmic and silver impregnations is but a mass of precipitated osmium or silver on surfaces and spaces pre-existing in the cell. The reality is a system of spaces with their enveloping membranes revealed by the techniques of Holmgren, Bensley, Parat and Painleve, and Guilliermond, in other words the vacuome." Disregarding the very great possibility that Bensley is not discussing the same structure as Parat and Painleve (Beams, 1931) it is quite evident that since osmium and silver techniques do so clearly show the plant plastid, Bensley is quite incorrect in assuming that these techniques *only* bring into evidence the limiting membranes of cytoplasm and vacuoles. It is true that perhaps the osmium and silver are precipitated on the interfaces of differentiated regions of cytoplasm that go to form the heterogeneous unit of living matter—the protoplast. It is true that these blackened regions may not be visible in the living cell and are in that sense artifacts, but in the plastid, at least, they certainly indicate zones of cytoplasm which we have reason to believe are specialised centers of even greater chemical activity than the nucleus. Since frequently these same techniques when applied to the animal cell show similar structures, is it not most logical to conclude that the Golgi zone, too, is a specialised region of cytoplasm and not, as Bensley and Parat maintain, vacuolar?

The question then may logically be asked as to how we are to dispose of the vacuolar network which Guilliermond (1930) holds to be homologous with the Golgi zone, and upon which the concept of the vacuolar Golgi region is based. The answer is simply that Guilliermond is describing the plant vacuole, a typical plant structure which performs certain physiological functions, turgor and translocation, peculiar to the plant. For this reason we should neither look for nor expect to find structures corresponding to the plant vacuole in the animal cell. There may be vacuoles in the animal cell—indeed it is difficult to picture a cell without them; but since they perform other functions than do the vacuoles of the plant cell they must be different in form and shape from those of the plant cell.

There is, undoubtedly, some resemblance between the Golgi zone and certain stages of the plant vacuole (Guilliermond, 1930) but a little serious consideration of their nature shows clearly that this similarity is very superficial. In the first place the form of the plant vacuole in elongating tissue, an open slightly anastomosing net, is very far removed from the complicated Golgi structure observed in glandular tissue. Secondly, according to the classical concept of the Golgi apparatus and emphatically stated by Bensley (1929) in his "vacuome" hypothesis, the osmium is precipitated in the cytoplasm of the cell; whereas, according to Guilliermond (1930) it is the vacuolar material itself which precipitates the osmium in the plant cell. Thirdly, in gland cells, at the height of secretion, the secretory droplets are plainly visible in the interstices of the blackened platework. On the other hand, the vacuome which forms the blackened network is itself the secretory product of the plant cytoplasm. In other words, in the animal cell, the osmiophilic substance plays a part in the elaboration of the secretory product, in the plant cell the osmiophilic vacuome is the secretory product. Obviously the two structures cannot be comparable. Fourthly, neutral red does not stain the Golgi material. This has been clearly shown in numerous instances. Special mention may be made of O'Leary's (1930) experiments on the living mouse pancreas and the work of Beams (1930) in demonstrating both the Golgi apparatus and the neutral red vacuome in the same cell. Fifthly, not only the network vacuome but the large vacuoles of the plant cell are frequently formed by the flowing together of small granules. That such a phenomenon does not take place in the case of the Golgi body is very evident from the failure of Covell and Scott (1928) to cause neutral red granules to flow together under the influence of osmium; from O'Leary's work on the mouse pancreas, and from Krjukowa's (1929) and Beams and Goldsmith's (1930) demonstration of the incorrectness of Parat's (1928) interpretation of the secretory cycle of the chironome larva. Sixthly, to the writer's knowledge Dangeard (1923), Guilliermond (1930), and Scott (1929) are the only investigators who have succeeded in demonstrating a blackened or neutral-red-staining network form of the plant vacuole. In the many vital experiments carried on by Weber, Martens, and Bailey and Zirkle, presumably in all of Bowen's osmicated plant material, and in several

thousand of the writer's preparations no network structure of the vacuole has been reported. It seems that this is a most pointed suggestion that the vacuolar network must either be a rather transitory stage, only existing in certain periods of development not handled by the above mentioned group of investigators, or else it must be strongly influenced by fixation. Seventhly, there is a marked difference in the period of life of the individual organism when the plate or network structure of Golgi and vacuome are visible. In the case of the animal cell the platework is most commonly observed in fully differentiated gland cells; whereas the network vacuome is almost entirely confined to that period of the life history of the plant when the meristematic tissues are elongating and large vacuoles are forming. Eighthly, the dictyosome formation, the secretory cycle, and the acrosome formation so characteristic of the Golgi body are conspicuous by their absence from the concept of the plant vacuole. Indeed the vacuole of plant material is apparently not even present during sporogenesis and spermatogenesis.

There seems to be only one conclusion permissible from this array of facts—the plant vacuoles are not comparable in the slightest degree to the Golgi zone. The vacuoles may possibly correspond to the secretory products of the animal cell or they may be comparable to the neutral red granules, or they may be an especially adaptive structure correlated with cell turgor and the means by which the plant maintains its rigidity, and thus have no counterpart in the animal cell.

The Cytoplasmic or Enzymatic Factor in Photosynthesis

I have hitherto spoken of the plastid as though starch were the direct product of the action of the cytoplasm of the plastid. In doing so I have ignored the various physiological aspects of photosynthesis because I felt that our knowledge of plastid structure hardly allowed for more than a very general statement, from a cytological point of view, of plastid activity. In thus considering the action of the plastid I have compared the carbohydrate end product of photosynthesis with the secretory product elaborated by the Golgi zone.

Owing to the close correspondence between the fixed pictures of the Golgi apparatus and the plastid, together with certain well established facts of photosynthesis, I should like here tentatively to modify this concept. It has been shown that in addition to chlorophyll and sunlight some additional cytoplasmic factor, in all likelihood of an enzymatic nature, is necessary for the production of starch. May it not be, then, that at least one of the direct products of the plastid cytoplasm is an enzym? If this should be the case there would be a harmony of structure and function between the animal and plant cell. Both possess, in addition to the already well established structures, regions of cytoplasm which upon fixation presents corresponding platework forms. During meiosis and spermatogenesis these specialised regions of cytoplasm have a very similar history. In function they both

elaborate enzyms. The greatest difference in the two would lie in the fact that through some photochemical reaction, as yet not understood, the plastid is enabled to utilise the energy of the sunlight to build up the simple sugars.

The most serious objection to be raised against the idea of the essential similarity of plastid and Golgi body lies in the fact that certain plants do not possess plastids. I wish at this time only to raise the question, and to suggest that its solution lies, not in searching further for osmiophilic substances in chlorophyll-less plants, but in a better understanding of the physiological functioning of the plant and animal cells.

Summary

1. A careful comparison has been made between the structure of the Golgi apparatus of various gland cells with the bryophyte plastid, and of the appearance of the Golgi body and the plastid in relation to the amount of the secretion or starch present.

2. The concept of the homology of the Golgi apparatus with the plant vacuole is reviewed in detail.

3. The results of these studies materially strengthen the theory that if the plant cell possesses a structure comparable to the Golgi apparatus, there exists strong evidence that this structure is the plastid and not the plant vacuole, as has been maintained. In support of this theory the present paper emphasizes the following points:

a. The essential similarity of the fixation pictures of the plastid and of the Golgi body. They are both present either as masses of differentiated cytoplasm which is traversed by numerous chromophilic plates, thus forming a platework in the interstices of which the starch or secretion is deposited; or they appear as differentiated masses of cytoplasm partially surrounded by a heavy staining ribbon or filament. Similar variations of these two typical fixation pictures are found to occur in both structures.

b. The similarity in the structure of the two bodies in relation to the amount of secretory material or starch present within the cell. When no starch or secretion is present both plastid and Golgi apparatus frequently appear as evenly staining masses of differentiated cytoplasm. With the appearance of some secretion both structures are more or less vacuolar. They are both complicated plateworks when at the height of activity and upon becoming gorged with the products of elaboration the platework seems to disintegrate.

c. The similarity in the function of the two structures; both are very probably the centers of enzym elaboration.

d. The fact that in many important instances the concept of the plant vacuole differs materially from that of the animal "vacuome-Golgi apparatus" hypothesis, thus clearly indicating that the plant vacuole and the Golgi apparatus are by no means comparable.

4. Since osmium and silver are precipitated around and within the plastid

—a cytoplasmic structure—theories maintaining that these metals are only precipitated on cytoplasmic membranes bordering upon watery vacuoles are ostensibly incorrect.

5. It seems that this similarity of structure and function between plastid and Golgi body, together with the demonstration of the fact that osmium and silver are precipitated within and upon cytoplasmic structures, should afford conclusive evidence that the classical concept of the Golgi body as expressed by Nassonov, Bowen, Gatenby, Morelle, Hirschler, Beams and others is correct. This concept is that the Golgi apparatus is a specialised cytoplasmic structure performing a definite function in the metabolism of the cell.

I wish to acknowledge my indebtedness to both Professors E. B. Wilson and H. W. Beams for their kindness in sending me the Golgi preparations I have used in this study. I owe much to Professor L. W. Sharp for his kindness in allowing me the use of his own research microscope and for his continued interest in the progress of the work. I have been greatly aided by the assistance of Katherine Weier in preparing all of my botanical material. This investigation was aided by a grant from the Committee on Grants-in-aid of the National Research Council.

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DESCRIPTION OF PLATES

All the figures were drawn with the aid of a camera lucida using in all cases a Zeiss apochromatic objective 1.5 mm. (num. aper. 1.30). Different compensation oculars were used in outlining the drawings so that their magnification varies to a certain extent.

PLATE XLVI

FIG. 1. A section of a spinal ganglion cell from the grasshopper. The Golgi apparatus is present in the form of discrete dictyosomes. From a preparation of Beams.

FIG. 2. A cell from the archesporial line of *Polytrichum commune*. The plastid bears a resemblance to the Golgi zone which cannot be without significance. Benda fixation, iron alum haematoxylin stain.

FIG. 3. The Golgi apparatus in the intestinal cell of the rat frequently is a curved ribbon with its borders quite heavily impregnated. It appears to enclose a region of differentiated cytoplasm. From a preparation of Beams.

FIG. 4. The plastid in the archesporial line of *Polytrichum commune* also frequently appears as a curved ribbon or thread with its edges heavily blackened. Kolatchev.

FIG. 5. In the Golgi apparatus of the rat intestinal cell cases are met with in which the blackened borders are quite fragmented. From a preparation of Beams.

FIG. 6. Upon occasion the plastonema of the archesporial plastid may also exhibit a fragmentation of its blackened borders. Kolatchev.

FIG. 7. The blackening of the regions bordering upon the chromophilic portion of the Golgi apparatus may be so reduced that this portion of the apparatus is delimited from the remainder of the cytoplasm only by very delicate blackened lines. From the same section as figures 3 and 5.

FIG. 8. The plastid may also be delimited from the surrounding cytoplasm by very delicately blackened regions. Kolatchev.

FIG. 9. In addition to the blackening along the edges certain regions within the chromophilic portion of the Golgi apparatus have reduced the osmium forming a tressil-like structure. From the anterior lobe of the hypophysis of the rat. From a preparation of Beams.

FIG. 10. Both the antheridial and somatic plastids of *Polytrichum commune* exhibit a fixation picture fairly similar to that shown in figure 9. The illustration here was taken from a somatic cell. Kolatchev.

FIG. 11. A fairly typical apparatus early in the secretory cycle. Pancreas of cat. From a preparation of Bowen.

FIG. 12. Illustrating how the plastid may correspond to the Golgi apparatus of the pancreas cell. Both are clearly regions of cytoplasm in which osmium or silver are deposited in the form of undulating plates. Da Fano.

FIG. 13. Golgi apparatus in the paratoid gland of the cat. The secretory granules are clearly visible in the interstices of the blackened platework. From a preparation of Bowen.

FIG. 14. The single large plastid of *Anthoceros*. After osmification as well as after other fixations, it appears as a complicated, but apparently regular, platework. In the interstices of this platework polarized light shows the presence of starch, represented here by dark patches. Kolatchev.

PLATE XLVII

FIG. 15. A plastid from the sporophyte tissue of *Anthoceros*. The regular platework has apparently disintegrated so that a few pieces separate the starch grains. Benda fixation, iron-alum haematoxylin stain.

FIG. 16. The Golgi apparatus near the end of a secretory cycle in the paratoid gland of the cat. The platework has broken down into discrete pieces which wind in and out between some of the secretory granules. From a preparation of Bowen.

FIG. 17. The Golgi apparatus in the caput region of the cat epididymis. From a preparation of Bowen.

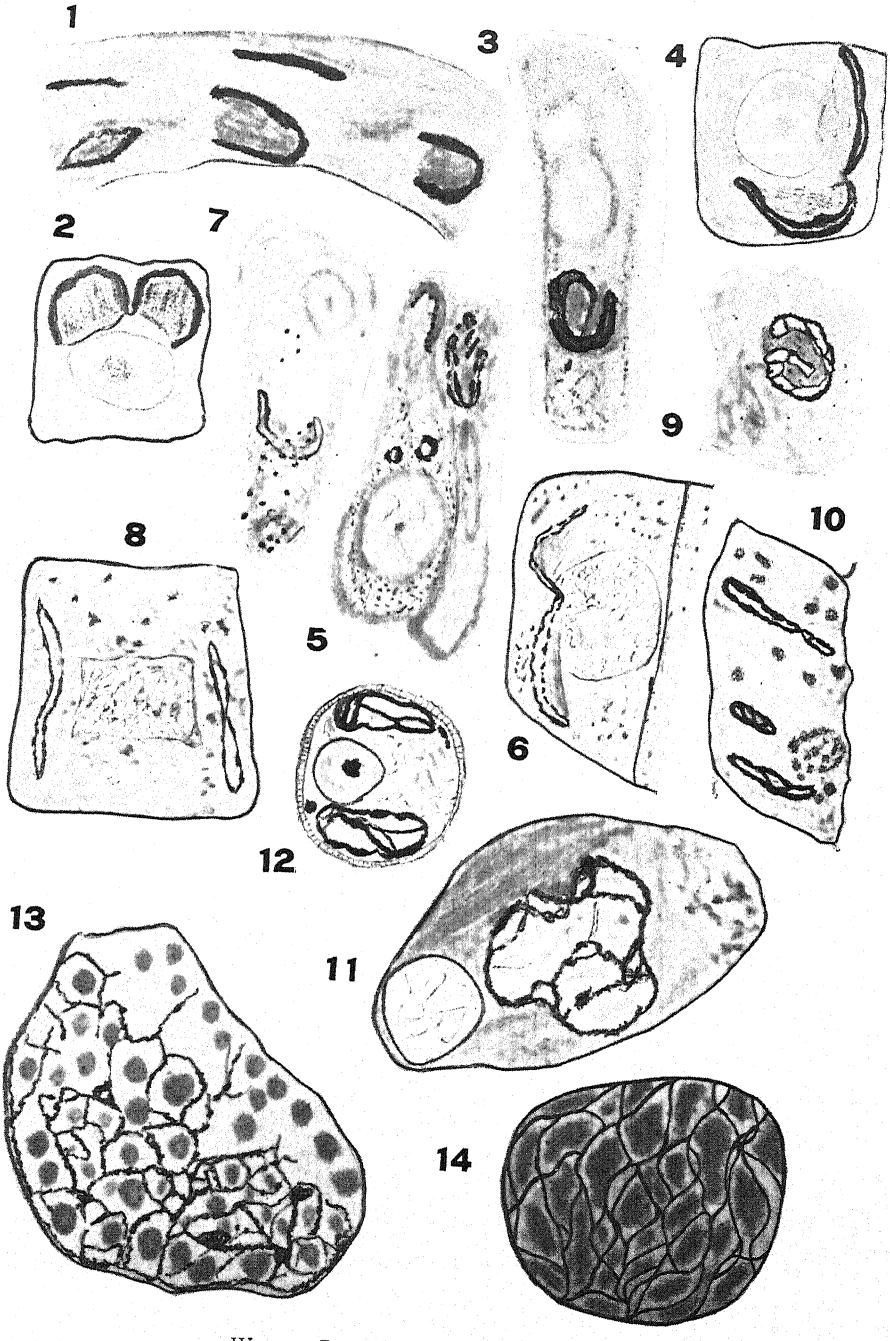
FIG. 18. A plastid taken from the archesporial line of *Polytrichum commune*. Kolatchev.

FIG. 19. A non-starch-containing plastid from the thallus of *Anthoceros*. Kolatchev.

FIG. 20. Plastids from *Elodea canadensis*. Benda fixation, iron-alum haematoxylin stain.

FIGS. 22 and 23. Non-starch-containing plastids from the thallus of *Anthoceros*. Kolatchev.

FIG. 24. A plastid with "vacuoles" which under polarized light are shown to contain starch. Kolatchev.



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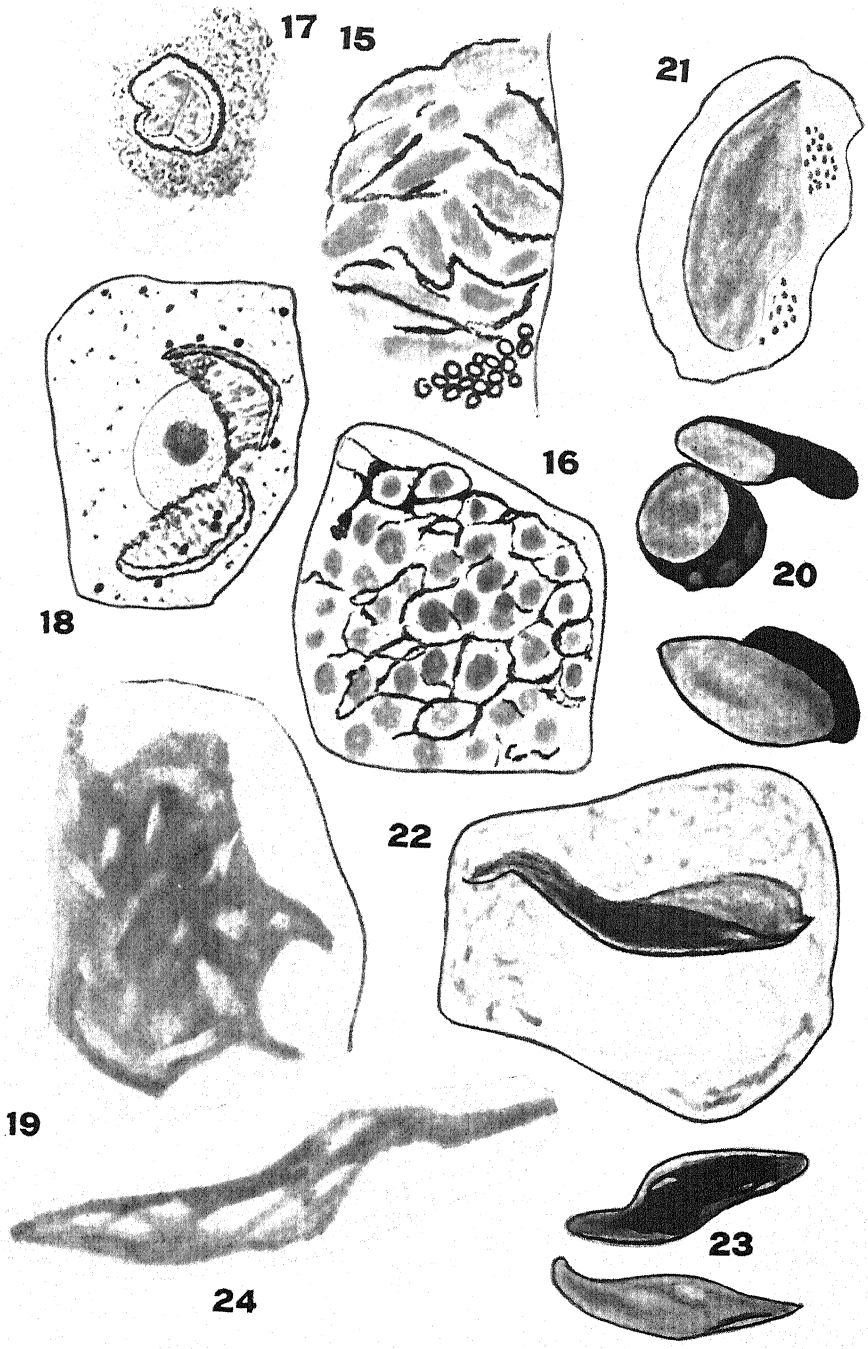

WEIER: GOLGI BODIES AND PLASTIDS

SOME FEATURES OF THE ANATOMY OF *FOUQUIERIA SPLENDENS*

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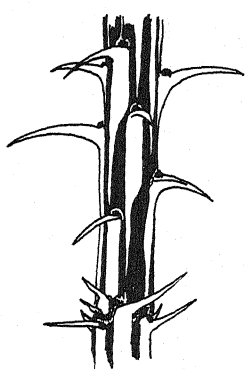
Fouquieria splendens, ocotillo or candle bush, with its switch-like thorny branches, two to five meters in height, its recurrent short-lived foliage and flaming inflorescence, is a familiar and striking form of the south-western desert areas. From the morphological and taxonomic standpoints, it has been fully described (1, 2, 3), and certain aspects of its physiology have also been discussed (4, 5); but with regard to the detailed anatomy of the genus, I have been unable to find anything but a few scattered references (6).

In the present paper, the structure of the plant as a whole is briefly reviewed, and certain characteristic features, such as the development of the corky layers and the distribution and structure of the peculiar water storage cells present in the cortex, are described in greater detail.

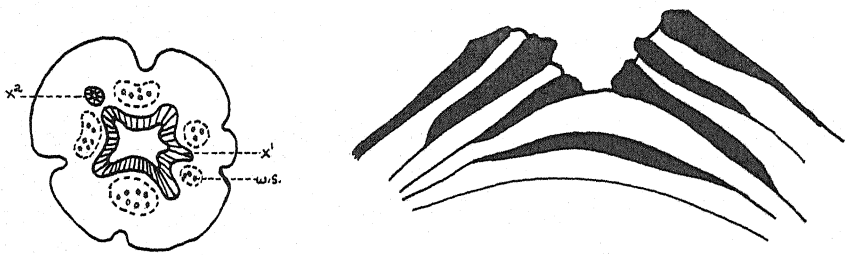
The striking appearance of the stem of the ocotillo (text fig. 1) is due to the reticulate pattern formed by the corky layers. The smooth hard spines which originate from the petioles (2, 3) are spirally arranged, and pass



TEXT FIG. 1. Habit sketch (× 1½) of leafless branch, showing spines, axillary buds and terminal bud scars.

downward at their base into the tapering lenticular ridges of the stem. In the current shoot, these islands of smooth cork are separated by a continuous network of furrows, and the outline of the stem, as seen in transverse section, is therefore sinuous (text fig. 2). Later on the furrows are filled out and broadened, until they first of all equal, and finally surpass the ridges in extent, and so form the conspicuous reticulum of ragged cork of the older stem.

This characteristic pattern, as might be expected, is repeated in the distribution of the underlying tissues. Thus, when the cork is removed, it is seen that the photosynthetic tissue beneath is stamped with the same design. A continuous network of darker green follows the course of the ragged cork, and represents the maximum thickness of chlorenchyma. The intervening mesh-areas, shaded by the dull brown of the thorn bases and their ridges, are lighter green in colour, and the assimilating tissue here is only half as deep. They



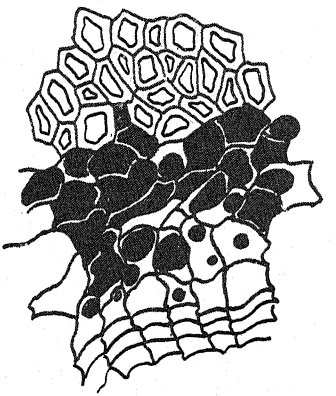
TEXT FIG. 2. Transverse section (diagrammatic) of a current shoot segment, showing the outlines of the stem surface, the stele (x^1), the axillary bud vascular supply (x^2), and the position of the water storage cells (*w. s.*) in the cortex.

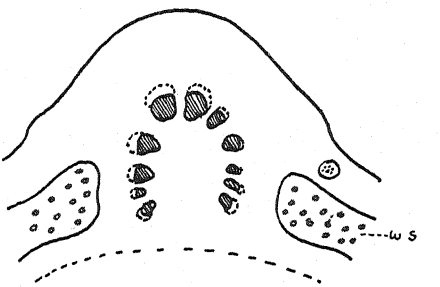
TEXT FIG. 3. Transverse section of laminated cork. Fibrous cork, clear; suberized cork, shaded.

are further distinguished by a definite horizontal septation, particularly noticeable in the older shoots (6). The reticular pattern is again repeated in the distribution of the water storage cells of the cortex. These cells are visible to the naked eye in a gross transverse section of the stem, where they appear as small pellucid circular or elliptical areas in the cortex underlying the stem furrows; that is to say, they occur in the same sectors which comprise ragged cork, stone cells, and the maximum development of photosynthetic tissue (text figs. 2, 5, 6).

The shoot of ocotillo is dicotyledonous in type, with normal secondary thickening (6). The epidermis of the current shoot is very soon replaced by cork, which originates from a cork cambium in the outer cortex. This cambium is at first discontinuous in activity giving rise at the beginning to the stem ridges, and only later to the ragged cork of the furrows. In the ragged cork of the older shoot a very distinct lamination is at once apparent, which is bound up with the intermittent seasonal growth characteristic of the genus. Leaf formation, as has been pointed out (4, 5), depends directly on water supply, so that, under natural conditions in the field, the number of crops of leaves per year is the same as the number of intermittent rains. The rapid unfolding of the new leaves is naturally accompanied by certain structural changes in the stem, such as the splitting of the older superficial cork, and its replacement by new suberized cell layers, and also by the further development of vessels in the xylem. The appearance of lamination in the cork is due to the juxtaposition of two different types of cells (text figs. 3, 4).

At the beginning of each burst of growth, the cork cambium opposite the ragged cork becomes intensely active, and gives rise to four or five rows of thin-walled cells. Fatty substances accumulate within these cells, impregnate their unthickened walls, and finally harden into a compact layer of suberin,

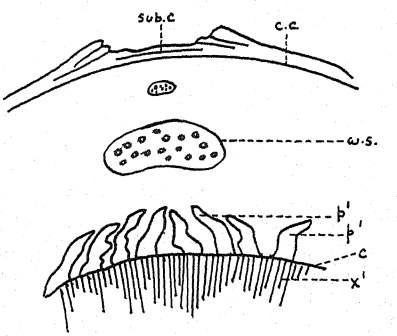




TEXT FIG. 4. Detail of figure 3, showing the junction of fibrous cork (above) and the unthickened cork cells of the early growing season (center) developing from the cork cambium (below).

TEXT FIG. 5. Transverse section of portion of stem (diagrammatic), showing the beginning of the axillary leaf vascular supply (center), and the position of the water storage cells (*w. s.*).

which stains brilliantly with Sudan III. As fat and suberin formation begin to slow down, carbohydrate metabolism supervenes, and a typical fibrous cork is now developed, not along the furrows only, but continuously beneath the ridges all around the circumference of the stem. In tranverse section the cells of this cork are thin walled and roughly rectangular in outline. As is seen from micro-chemical tests only the middle lamella is suberized, and the wall thickening is due to a heavy lining of cellulose. When macerated, or



TEXT FIG. 6. Transverse section (diagrammatic), showing the orientation of the phloem groups with reference to the water storage cells. *x*, xylem; *c*, cambium; *p*, phloem; *w. s.*, water storage cells; *c. c.*, cork cambium; *sub. c.*, suberized cork.

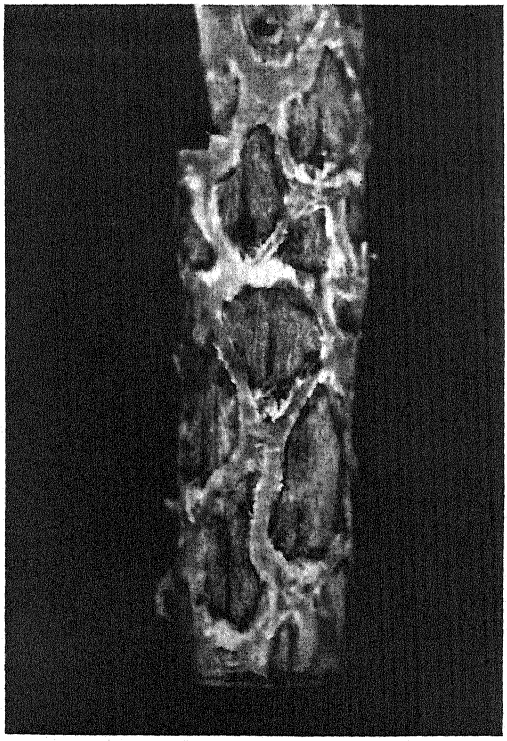
when seen in longitudinal section, the cells appear as short fibers, pointed at both ends, with occasional narrow pits, here and there, obliquely on the cell walls. As the water supply of the plant becomes exhausted, growth slows down and eventually ceases, and the leaves are finally shed. With the onset of the next rain the whole process is repeated; the overlying corky layers are split, and the result is the familiar raggedness of the furrow bark. The zone of ridge bark is comparatively narrow, and the lamination is much less obvious. The tissue here is made up entirely of the above described fibrous cork cells, which vary slightly in size at the beginning and end of the growth period.

Next to the cork cambium lies the cortex, which includes collenchyma, stone cells, photosynthetic tissue, and water storage cells. The collenchyma forms an inconspicuous ring one to three cells in depth. Isolated groups of stone cells occur in the cortex beneath the ragged cork. The main bulk of the cortex is taken up by a photosynthetic zone, which varies in depth beneath the smooth and ragged cork, and which passes gradually into typical cortical parenchyma. Throughout this tissue there ramifies a continuous network of water storage cells. On carefully dissecting away the surrounding green cells, the reticular path of the water-storage cells is at once exposed. The reticulum follows the line of distribution of the ragged cork, as is apparent in the photograph of the dissected stem (text fig. 7). The nodes of the network coincide with the actual nodes of the stem, so that the leaf axils are surrounded by water storage tissue. When examined under the microscope, the cells are easily distinguishable by their larger size, and also by their contents. The reaction with Sudan III points to the presence of fatty substances throughout the stem as a whole, but the water storage cells are conspicuous in that each contains a single large drop of oil, often approximately equal in diameter to the cell nucleus. Smaller droplets are only rarely observed. The cell walls remain uncolored, in marked contrast to the deeply stained, and therefore fat-impregnated, walls of the surrounding tissues. Further differences in content are seen on the application of Fehling's test (7), and also of iodine-potassium-iodid. With the former a deep copper-red precipitate indicates the presence of reducing substances, presumably sugars, which are conspicuously lacking in the neighboring cells. The iodine test, as might be expected, reveals an almost complete absence of starch grains in these sugar-containing cells, while in the remainder of the cortex, particularly in the outermost layers and also next to the phloem, starch is very abundant. It is highly probable that the distribution of fat, sugar, and starch as here described is not constant throughout the growing periods or through the year as a whole.

The stele is of the usual dicotyledonous type (6). In transverse section, the phloem cells appear as irregular triangular wedges, separated by the centrifugally broadening rays, the apices of the triangles in general converging toward the water storage cells. This convergence is in line with the general

reticular pattern of the stem as a whole (text fig. 6). Seasonal growth rings are obvious in the xylem and in general equal the number of the laminae of the cork. The cells which make up the vascular cylinder are in no way unusual in type. Lateral sieves are present on the sieve tubes. Border pitted vessels (6) and fibers make up the bulk of the xylem. Horizontal septation of the surrounding cortex and of the enclosed central pith is observed (6).

The vascular supply of the axillary leaves is depicted in text figure 5. Composite strands of xylem and phloem spring off from the central stele,



TEXT FIG. 7. Photograph of dissected stem, showing the reticulum of water storage cells (× 1).

ramify, and diverge upwards and outwards towards the base of the spine and the leaf axil. In transverse section they appear first in a horse-shoe, and at a higher level in a ring. The water storage cells are seen to be in close connection with the vascular branches. Isolated groups of stone cells are also abundant in the leaf axils (text figs. 5, 6).

The structure of the root is in no way unusual. Lamination of cork is present here, as in the stem, but the cork cells are not of the fibrous type. In the older root water storage cells do not appear in the disintegrating cortex. The youngest stages of root development, owing to present lack of material, have not been examined.

The outstanding physiological character of the desert ocotillo is its extreme promptness in producing leaves immediately after an increase of the water supply. Such an habit implies very efficient water conduction and high meristematic activity, the latter necessarily dependent on a readily available food supply. Cannon (4) states that forty-eight hours are sufficient for the bare branches of an ocotillo shrub to become completely clothed with leaves. The network of water storage cells, since they contain an abundance of water, oil, and sugar, and since they are in close proximity to the nodes, presumably function in this connection, and directly facilitate this rapid development.

Summary

The anatomy of the stem of *Fouquieria splendens* is that of a typical dicotyledon, but possesses certain unusual features.

The characteristic pattern of the stem surface is due to the reticulate distribution of smooth and ragged cork. The former is made up of fibrous cork cells only, while in the latter, markedly laminated layers of non-fibrous heavily suberized cork cells alternate with the fibrous cells. The heavily suberized layers are developed at the first onset of the growing period.

The maximum thickness of the underlying cortical chlorenchyma follows the reticular distribution of the ragged cork, and beneath this tissue lies the network of water storage cells with their reserve of water, oil, and sugar.

The distribution of the water storage cells and their content of reserve material are probably important factors in the rapid development of the foliage of this species after an increase of the water supply.

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A NEW CYCADEOID FROM NEW JERSEY

M. A. Chrysler

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Source

The material which forms the basis of this account was collected in January 1930 at the Hampton Cutter clay pit at Woodbridge, New Jersey, by Mr. Wm. H. Koch, a member of a party from the Geological Department of Rutgers University. The fossil was submitted to Professor A. O. Hayes of the Department of Geology, who very kindly turned the specimen over to the writer for purpose of identification (Chrysler, 1931). It is a pleasure to acknowledge my indebtedness to these two workers.

The Hampton Cutter pit is well known to paleobotanists, for it furnished many of the fossil leaves which formed the subject of Newberry's monograph of 1895 on the Flora of the Amboy Clays (Newberry, 1895). A large amount of clay has been removed from this pit, and in recent years few leaf impressions have been found, but instead a large number of pieces of lignitic wood, small branches, cone-scales and seeds. Within the past few months however fossil Angiosperm leaves have again been found. This is probably explained by the varying Cretaceous topography of this large pit. The active interest and cooperation of Mr. Hampton Cutter, owner, and Mr. Roy E. Anderson, superintendent of the pit, have been of much service to botanists and geologists alike, and their help is gratefully acknowledged.

The geological horizon represented in the Woodbridge pits is the Raritan formation of the upper Cretaceous, which here rests directly upon the red shales of the Triassic period. From evidence supplied by molluscs, the Raritan formation is believed to be an estuary deposit (Newberry, 1895, p. 22). The recent discovery of dinosaur tracks at Woodbridge confirms this view. The clay deposits in question outcrop in a belt extending from the Delaware River to Raritan Bay, thence across to Staten Island, where the material was obtained for the monograph on Cretaceous conifer remains by Hollick and Jeffrey (1909).

External Features of the Fossil

The specimen as received by the writer consists entirely of lignite with a few imbedded pieces of pyrite, and represents a short apical region of a trunk with attached leaf-bases. The drying up of the specimen had already caused it to break into a larger lower and small upper portion (text fig. 1), and the leaf-bases were beginning to split up. The general appearance is shown in

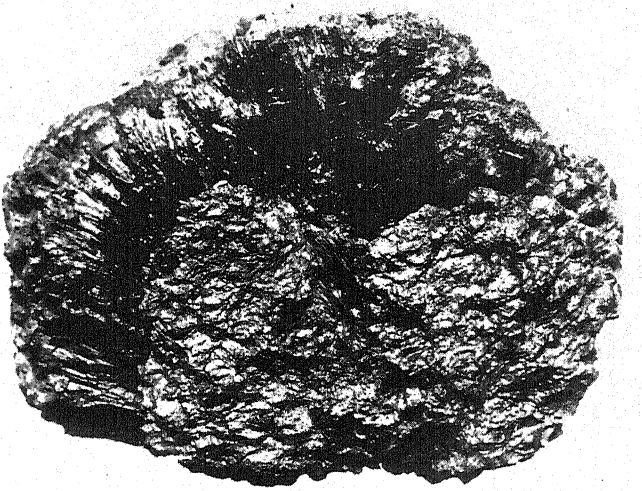
text figure 1, representing a view from above, and figure 2, taken from below. The latter clearly shows that only about half of the horizontal extent of the trunk is preserved. The fossil has a diameter of 180 mm. The friable nature of the material indicates that the tips of the leaf-bases have been worn away, so that the original diameter of the specimen was probably well over 200 mm. The diameter of the trunk proper is 85 mm, while the height of the fossil is 110 mm over all.

Finding that the specimen had excited considerable interest, Mr. Koch returned to the clay pit in the course of a few days, and secured further material at a distance of not more than three feet from the location of the earlier discovery. The latter collection consists of about a dozen detached pieces, some of which are represented in text figure 3. It was at once recognized that the state of preservation of these pieces is superior to that of the larger fossil, and they form the basis of much that follows. Evidence for considering that they are detached leaf-bases from the same trunk as the large specimen will be presented later. These pieces measure about 50 mm long by 18 mm wide; they are 4 mm thick along the median ridge, and thin off at each edge, so that a transverse section (text fig. 5) shows a more or less rhomboidal or spindle shape. What appears to be the basal end of the pieces is rather flat or scale-like, while the outer or distal end is more quadrangular.

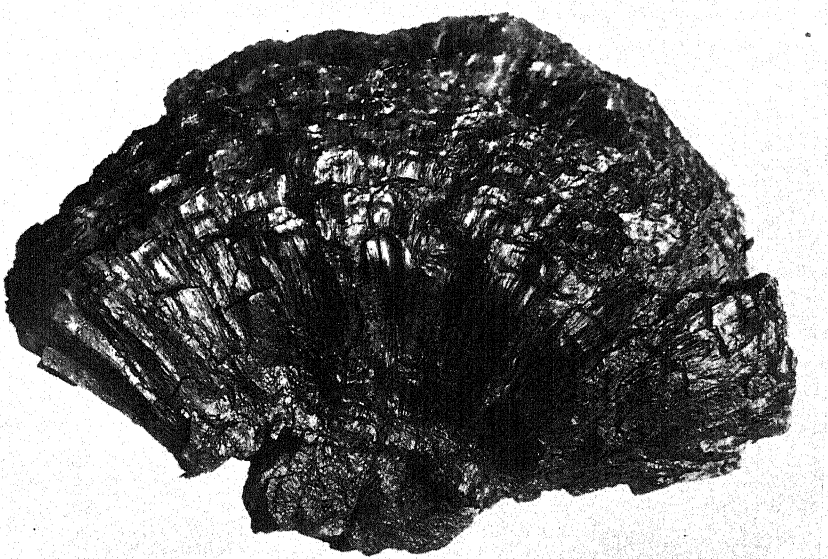
The scale-like leaf-bases forming part of the large specimen bear evidence of having been much flattened and deformed through pressure. A number of them have been pried off, but the periderm formation to be described has resulted in the splitting off of flake-shaped fragments, rendering the original form hard to demonstrate. Certain superficial markings among the leaf-bases suggested that they might be the apex of fructifications. The most promising of these was removed, imbedded and sectioned, but no evidence of a reproductive axis with sporophylls or seeds could be found. It is concluded that these regions are merely leaf-bases in which the periderms have cut off more or less concentric lamellae, so that no evidence has been found against the opinion that the specimen was not yet producing fructifications at the time of fossilization. It is of course possible that only the apical region was sterile, and unfortunately that is the only region represented in our specimen.

Methods of Study

Since the material is definitely lignitic, and shows only scattered deposits of pyrite, the celloidin method (Jeffrey, 1917) is applicable. After a thorough treatment with hydrofluoric acid the leaf-bases were slowly infiltrated with celloidin and softened in a mixture of glycerin and alcohol. It was found possible to cut some of the sections as thin as seven microns. Chlorine water was used to bleach sections, but it was found that when sufficiently thin

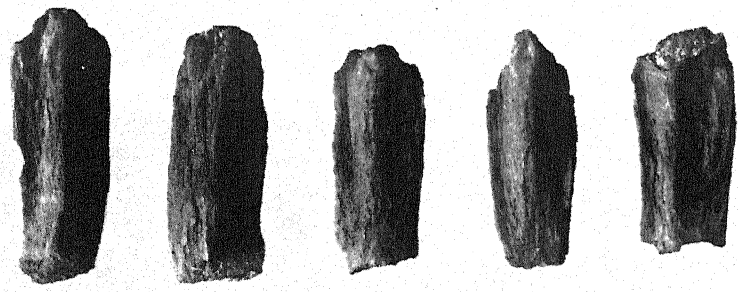


TEXT FIG. 1. View from above, showing leaf-bases worn away at various angles. The two pieces, into which the specimen broke as a result of drying, can readily be seen. × 2/3.



TEXT FIG. 2. View from below, showing portion of axis as well as lower surface of a number of leaf-bases. × 2/3.

they showed detail quite well if dehydrated and mounted in euparal. In photographing the sections, the value of a green filter passing light of 4600–6000 Å.U. was amply demonstrated.



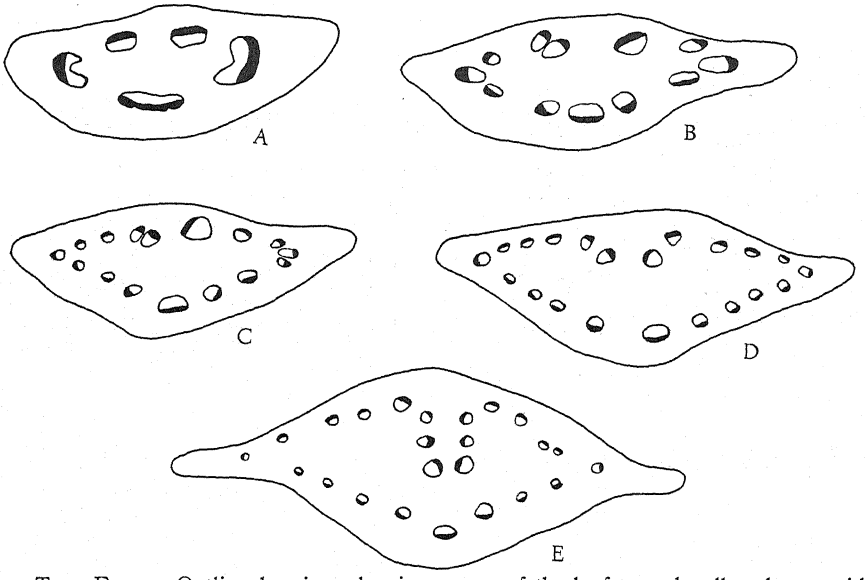
TEXT FIG. 3. Some of the detached leaf-bases found near the large specimen. × 3/5.

STRUCTURE OF THE LEAF-BASES

Course of the Bundles

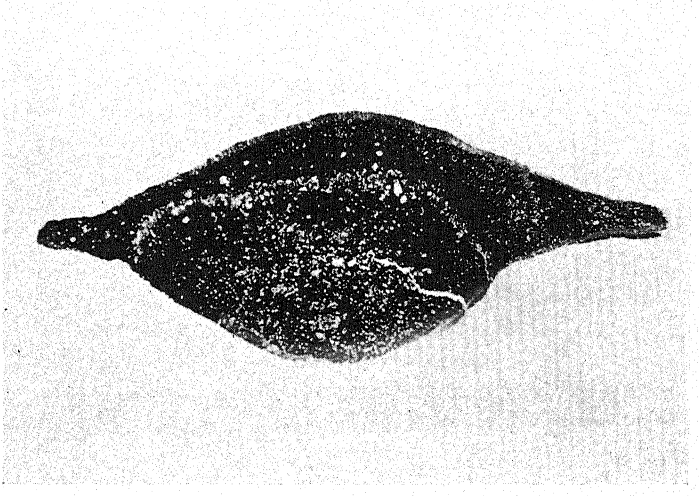
Serial sections have been cut through several leaf-bases, and from these it has been found possible to trace the characteristic method of branching of the bundles. In the sections taken nearest the base of the petiole five bundles are present (text fig. 4 *A*); this condition will later be referred to as stage III. These bundles are: a rather flat dorsal (abaxial) strand, two stout fan-shaped laterals, and two ventral strands. In each of these the xylem faces the middle of the leaf-base, and there is a considerable layer of secondary xylem. Very soon the dorsal strand splits off a small branch from each edge (as Lignier says, it "trichotomizes"); this represents stage V. Soon afterward each of the laterals likewise splits into three. At a point somewhat further out each of the two ventral strands "dichotomizes," rotating meanwhile slightly toward the centre of the petiole (fig. 4 *B*). One of the ventral bundles may lag behind the other in its splitting, as is evident from the figure. Each of the laterals next trichotomizes a second time (fig. 4 *C*). This process occurs in general yet a third time (fig. 4 *D*). It is obvious that in this region of the leaf-base the activity is restricted to the lateral strands. No further division of the dorsal bundles occurs, but somewhat further outward (fig. 4 *E*) it may be seen that each of the four ventral strands has divided, and three of the resulting strands on each side of the median line have turned definitely toward the centre of the leaf-base. No further divisions of the bundles has been observed, and it is probable that about twenty-five bundles constitute the normal petiolar supply. The resemblance of the bundle

plan to that of *Bennettites gibsonianus,* as figured by Carruthers (1870, Pl. 60, fig. 7), is striking. Among the diagrams of petioles included in Wieland's



TEXT FIG. 4. Outline drawings showing course of the leaf-trace bundles, drawn with camera lucida. Xylem region of each bundle is left blank, phloem shown black. The dorsal (abaxial) side of the leaf-base lies below. Explanation in the text.

(1906) text figure 33, the Woodbridge specimen resembles *Cycadeoidea turrita* rather closely. The bundle plan thus establishes the claim that we are here dealing with a species of *Cycadeoidea.*



TEXT FIG. 5. Section through leaf-base at about the level of text figure 4*E*. Explanation in the text. × 5.

Structure of the Bundles

As appears in Plate XLIX, figure 13, the greater part of the volume of a bundle is occupied by secondary xylem, consisting of tracheids squarish in section, with a ray forming every second or third radial row. It has not been possible to make out any wood-parenchyma; Wieland makes no reference to such cells, and his figures show none. The region of the cambium can be made out, although this is badly disorganized; also the phloem, not all of which is secondary. There is no certain indication of phloem fibers, for it is difficult to distinguish between the brown contents of cells and a possible secondary wall when the contents does not altogether fill the cell-cavity. In the broad bundles found in the basal region of the petiole, little or no primary xylem can be made out, but an ill-defined mass occurs on the side of the secondary xylem next the pith. Figure 15 shows the median (abaxial) bundle from the distal region of the leaf-base. In addition to the usual mass of secondary xylem, there is visible on the inner face of this a group of elements making up the primary xylem, which is apparently centripetal, judging from the large tracheids along the inner edge. It will be noticed that the primary phloem is also particularly well developed. The marked resemblance of this type of bundle to the ones found in the megasporophyll of certain species of *Cycas* has already been pointed out by Wieland (1906, p. 65). Plate XLIX, figure 14, which is here introduced for comparison, shows a bundle from the basal region of the megasporophyll of *Cycas circinalis,* obtained at the New York Botanical Garden through the courtesy of the Director. The secondary xylem of this bundle is strongly developed, and has a small amount of centrifugal primary xylem applied to its inner face; separated from this by a zone of parenchyma cells is the much better developed centripetal xylem consisting of the protoxylem and a group of metaxylem elements which are the widest of the whole bundle. It was long since figured by Mettenius (1861) that in *Cycas revoluta* the proximal region of a leaf trace contains much secondary xylem, and as a bundle is traced outward the centripetal elements gradually increase while the centrifugal ones disappear. My own observations on *Microcycas* indicate the same sequence. The secondary xylem of the cycad petiole appears to be a feature carried up so to speak from the central cylinder of the stem for a short distance into the petiole, where it gradually disappears and is replaced by the centripetal xylem which is so characteristic of the rachis of a cycad leaf.

By employing sections of thickness from five to seven microns, it has been possible to make out with certainty the nature of the secondary wall of the tracheids. These elements are prevailingly marked by tightly wound spirals merging into scalariform thickenings. Narrow tracheids frequently show a single row of pits ranging from circular to ovoid and slit-shaped, and in some cases obviously bordered (fig. 9). A relatively small number show alternate pits which may or may not show a distinct border. No reference to pitted tracheids in the leaf-base has been found in the earlier accounts.

Carruthers (1870) and Lignier (1901) describe or figure their species as showing scalariform tracheids. In the xylem of the trunk of *C. Paynei* pitted tracheids are present, as shown in Wieland's plates (vol. 2, Pls. 34, 35). The situation appears to exactly parallel what is found in modern cycads, where the stem wood of most of the genera is characterized by pitted tracheids, while the petioles show mostly scalariform elements, in accordance with the law of conservative organs (Chrysler, 1926). But just as the genera of modern cycads differs from one another in this respect, the presence of pitted tracheids in the petiolar strands of the New Jersey specimen may point to its being a different species from the ones previously described.

In a few cases well-defined structures resembling tyloses have been observed, taking the form of proliferations extending from what are probably ray cells into an adjoining tracheid (Pl. XLVIII, figs. 7, 8). Tyloses extending from ray cells into tracheids of *Pinus* have already been described by the present writer (Chrysler, 1908). It is of course possible that these intrusions merely represent regions where fossilization has brought about a rupture of the wall of a tracheid through which the products of decomposition of the xylem protrude, although the appearance is very suggestive of tyloses.

Periderm

One of the most conspicuous features of our fossil is the layer of periderm which surrounds the leaf-bases and sends branches into the interior. The general appearance of these layers is shown in text figure 5, where a band of periderm may be seen along the upper edge of the section, also along the lower right edge. The right wing is being cut off by an internal layer. Arising near the lower left edge is another layer which traverses the section in a curve and again joins the lower edge toward the right of the section. The lower left region of the section is not provided with periderm, but there appears instead a layer of ramenta, to be described later. On the lower side of the right edge is a dark line, representing a patch of ramenta external to the periderm. The superficial layer occurs at all levels of the leaf-base, being visible in the sections cut from the proximal region, and extending to near the distal end, where the state of preservation becomes unfavorable. The internal or invading layers become noticeable only at some distance from the base, for instance at about the level represented in text figure 4 *D*, but from this point outward they are frequent. Their course varies from one level to another, and the general plan differs in different leaves. As would be expected, these internal layers arise subsequent to the formation of the other tissues; this is shown by the fact that they pass through vascular bundles. It is apparently due to these invading periderms that the attached leaf-bases are so seriously broken up into scale-like portions. To what extent this breaking up of the leaf-bases occurred in nature would be hard to say, but it is a matter of common observation in modern cycads such as *Dioon* that the leaf-bases are cut off by successive layers of periderm (Chamberlain, 1911, p. 85). Both

the external and internal layers of periderm appear to be an unique feature of the Woodbridge fossil.

The thickness of the superficial periderm varies, and usually measures approximately 0.3 mm. The cells have the flattened form and radial arrangement characteristic of phellem, and are in as many as sixteen layers. The walls have suffered some distortion, but are otherwise well preserved; they are usually arched outward. In some cases the original cuticle of the petiole may clearly be seen, as in figure 17, where it appears as a broad light-colored line between periderm and ramenta. The cells lack the dark brown decomposition product which fills the cells of the fundamental tissue, but frequently a large number of small granules are present.

Ramenta

Covering the leaf-bases, and still adhering even to the detached bases, is a well-defined layer of ramenta. These are uniformly one cell in thickness, and have a width of 10–12 cells, tapering off to a thin edge on each side (fig. 12). The walls are light brown, and a dark brown substance may or may not occupy the cell-cavity. This is apparently a decomposition product similar to what occurs in the fundamental and vascular tissues. The thickness of the walls is fully twice that of the cells of the fundamental tissue. The ramental layer frequently lies outside the periderm, but may also be seen against the epidermis of the leaf-base in areas where the periderm is absent (fig. 12). It is probable that the number of cells in the thickness of a ramentum is a diagnostic feature of importance; *C. micromyela* like the Woodbridge specimen has ramenta uniformly one cell in thickness, while *Bennettites gibsonianus* and some of Wieland's species show ramenta several layers thick.

Stomata

These are best seen in the longitudinal sections. In regions where the epidermis is still intact there have been found pairs of thick-walled accessory guard cells curved toward one another and projecting slightly above the level of the epidermis (fig. 18); between these at a slightly lower level is the pair of guard cells whose walls are only moderately thickened. The stomata are covered by the ramental layer, and are of a xerophytic type, somewhat resembling the stomata of the leaf of *Cycas revoluta*.

Secretory Canals

Wide ducts of the schizogenous type are to be seen throughout the material (figs. 6, 11), and the state of preservation is frequently very good. While there is no actual evidence, it is probable that the ducts contained gum. They are generally filled with a decomposition product which in some cases is darker than that of the adjoining cells.

Fungus

Certain of the leaf-bases are found to be attacked by a filamentous fungus, which is in fossilized condition like the host, having the same brown color, or at times a smoky tint verging to black. The fungus occurs beneath the layer of ramenta (fig. 10), or occupies cell-cavities or the lumen of tracheids. From the color and position of the fungus, it appears probable that it was parasitic, but further inferences would be unwarranted.

Structure of the Axis

A sample of the axis of the large specimen was imbedded and sectioned. It was found that the condition of preservation was quite unsatisfactory, but it was possible to recognize the vascular cylinder and to make out the scalariform tracheids, together with very numerous short ray cells.

Discussion

It will now be in place to review the evidence for the opinion already expressed, that the detached leaf-bases actually belong to the larger specimen. First, the detached pieces were secured from within two or three feet of the larger piece. Second, ramenta and secretory canals are the same in both pieces. Third, the periderm with its habit of cutting off scale-like fragments is characteristic of both. Finally, the arrangement of vascular bundles is similar as far as can be made out, in view of the poor preservation of the attached leaf-bases. It seems safe to conclude that some leaf-bases became detached, and thereby enjoyed an advantage in the matter of preservation.

The arrangement of the vascular bundles of the leaf-bases, the structure of these bundles, the ramenta, and the secretory canals establish the identity of this fossil as a species of *Cycadeoidea.* A noteworthy feature is the preservation of the plant as a lignite, a condition which is practically unique among cycadeoids. An approach to this is seen in the example from Alberta, Canada, and is described by Wieland (1921) under the name *C. Sternbergii* thus: "cast and carbonized portions of a large armour fragment." Although it was not possible to prepare sections of the Alberta fragment, we have been exceedingly fortunate in being able to apply the celloidin method to the New Jersey specimen, and thus secure sections which can be readily studied under high power. Although much coniferous lignitic material has been secured from the Cretaceous deposits of Staten Island, N. Y., and described by Hollick and Jeffrey (1909), there are no previous records of the finding of cycadeoid trunks in this region. And yet the occurrence in the Woodbridge pits of leaflets referred by Newberry (1895) to *Podozamites* would suggest the eventual discovery of cycad trunks of some description. The statement of Berry (1910) may be quoted in this connection: "All of the Raritan species of *Podozamites,* in common with those from other localities and horizons, which are based on detached leaflets, are extremely unsatisfactory from the

standpoint of the botanist, since their true position remains doubtful." Reference to Newberry's figures (Pl. XIII) will show that the fragments of leaflets were deficient in clear diagnostic features, and may possibly have belonged to cycadeoids such as our specimen. Until the finding of the New Jersey specimen, the only cycadeoids reported from eastern North America were the ill-preserved cast from the Triassic of Prince Edward Island, named *C. abequidensis* by Dawson (1871), and the magnificent silicified trunks collected in Maryland in 1860 and succeeding years (Fontaine, 1889).

In view of the fact that the Maryland stations are only about two hundred miles from New Jersey, the question arises whether our specimen is the same species as any of the nine species recognized by Ward (1897, 1905) among the collections from Maryland. Bearing in mind Wieland's mature analysis of the "criteria of Cycadeoid species" (1916, p. 9), it appears clear that any expression on this point must be premature, because the criteria which have been adopted in distinguishing the Maryland species are mostly lacking in our specimen, while the plan of the vascular bundles and the structure of the ramenta, so plainly shown in our material, are not figured for the Maryland specimens. It is easier in fact to compare our leaf-bases with those of Dakota species, or especially with *C. micromyela* Mor., a silicified fossil from the Lias of Calvados, in which Lignier (1901) found the following condition: the leaf-trace begins as a simple strand (for convenience we will designate this as stage I) made up of a small central and two much larger lateral pieces which soon separate from the median (dorsal) portion (stage II). The laterals next divide (stage III), and each "dichotomizes" (stage IV), while the dorsal strand "trichotomizes" (stage V), thus giving rise to a system of eleven bundles. It has been shown in the context that the Woodbridge specimen differs from *C. micromyela* not only in having abouty twenty-five strands instead of eleven, but also in the method of branching of the leaf-trace. Up to stage III, in which five strands are present, both specimens behave alike, but following this there is in our specimen a pronounced difference in the behavior of the lateral and the ventral bundles, for while the latter fork or "dichotomize," the lateral bundles "trichotomize" repeatedly; that is, the laterals keep on splitting off small bundles from their flanks. Stage IV is thus variable according to the species concerned. Stage V, in which the dorsal strand splits off a small bundle from each flank, is found in both specimens. The extent to which the branches of the ventral bundles turn inward is about as great in our specimen as in any of the species figured by Wieland, and much greater than in *C. micromyela.*

Piecing together the various sources of information, it appears that in *Cycadeoidea* the leaf-trace begins as a single curved strand (stage I), which while still in the cortex divides into three (II); each of the outer strands divides, producing a curved row of five bundles (III). There is thus established a system consisting of a median (abaxial) bundle, on each side a lateral, and on the adaxial side a pair of ventral bundles. Each of these three

displays its individuality—the median bundle trichotomizes once, the laterals may or may not trichotomize several times, while the ventrals may dichotomize and in many species turn inward in marked fashion.

It is certain that the Woodbridge specimen is not *C. micromyela.* We cannot at present compare our specimen with the Maryland trunks. Among the Dakota species, Wieland's *C. turrita* has about twenty-five petiolar strands arranged much as in our specimen, but Wieland considers the status of *C. turrita* to be uncertain (1916, p. 93). *Bennettites gibsonianus* has a petiole built on about the same plan, but the ramenta are three cells thick. Moreover the very numerous and conspicuous layers of periderm in the New Jersey material appear to be an altogether unique feature. These differences appear to warrant the establishing of a new species for our fossil. It has been thought well to make the name indicate the most conspicuous feature. A condensed description follows.

Cycadeoidea peridermalis, n. sp.

Trunci apice brevi cum foliorum basibus, nonnullis segregatis; fructificationibus nullis; substantia lignosa; basibus foliorum prope recta linea ab axe trunci protendibus; sectione transversa rhombiforma; basibus foliorum periderma et exteriora et interiora exhibentibus; ramentis unius cellulae latitudinem habentibus; fasciculis circiter quinque et viginti; ligno fasciculorum secundario copioso, primario raro; tracheideis plerumque scalariformibus.

Short apical region of a trunk with leaf-bases, some of which are detached. Fructifications apparently absent. Material is lignite. Total diameter 180 mm, diameter of trunk 85 mm. Leaf-bases extend nearly at a right angle with axis of trunk, except the uppermost ones. Length of detached leaf-bases 50 mm, width 18 mm, thickness 4 mm at the middle, transverse section more or less diamond-shaped. All leaf-bases show in thin section both an external layer of periderm and several internal layers which eventually cut off irregular flakes, thus destroying original form of organ. Ramenta uniformly one cell in thickness. Secretory canals of the schizogenous type are abundant. Branches of the leaf-trace number about twenty-five, arranged parallel to surface of leaf-base, but the ventral ones turn strongly inward. All bundles show considerable secondary xylem, but little or no centripetal xylem. Tracheids mostly scalariform. A fungus, probably parasitic, is present locally in and upon the superficial cells of leaf-bases.

Occurrence.—Raritan formation, Cretaceous. Hampton Cutter clay pit, Woodbridge, Middlesex County, New Jersey.

Collection.—Department of Botany, Rutgers University.

The discovery of a cycadeoid in the Cretaceous of New Jersey raises anew the question of climate which was prevalent in this region during the period concerned. It is of interest to note that Wieland has summed up the implications of his extended survey of cycadeoid localities thus: "It is probable, however, that for Cretaceous time, the period of driest intra-continental climates is marked by the culmination of the cycadeoids. . . . No other fossil plants are more obviously xerophyllous." (Wieland, 1921, p. 80.) Refer-

ence is made here no doubt to the stem habit and leaf structure, both of which have been exhaustively described in Wieland's monograph. If the canals distributed through the leaf-base secreted mucilage, this may be cited as an additional xerophytic feature, while the profuse ramentum and the outer periderm layer may point in the same direction. As to the other plants found associated with the New Jersey specimen, some are impressions of dicotyledonous leaves with no structure preserved, while conifers of highly xerophytic type such as *Widdringtonites* (Hollick and Jeffrey, 1909, Pl. 5) were well represented. If the flora of the Woodbridge region is fairly indicated by the coniferous remains found by Hollick and Jeffrey in the adjacent pits of Staten Island, we may picture a region having more the aspect of a dry than of a mesophytic woodland. Evidence is not lacking to show that warm climates extended much further north than New Jersey during the Mesozoic, and the finding of a cycadeoid adds to the evidence for dryness.

Summary

1. A lignitic cycadeoid is reported from the Cretaceous of New Jersey. This specimen is unique in its location, method of fossilization, and in certain features of internal structure.

2. By means of serial sections the course of the leaf-trace bundles has been followed in the leaf-bases.

3. The structure of these bundles and of the unusual periderm layers is described, and it is concluded that this fossil constitutes a new species of *Cycadeoidea*.

4. A fossil fungus is associated with the leaf-bases.

The writer wishes to express his sense of obligation to Dr. Arthur Hollick of the New York Botanical Garden, and Professor G. R. Wieland of Yale University, for the assistance they have so kindly rendered during the progress of this work.

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EXPLANATION OF PLATES

PLATE XLVIII

FIG. 6. Region near edge of leaf-base; transverse section showing external periderm, also one of the internal periderms stretching across the section and connected with the external layer. Two of the gum canals are visible as large blank spaces. × 25.

FIG. 7. Detail of secondary xylem of a bundle; longitudinal section, showing scalariform tracheids, rays, and apparent tyloses. × 280.

FIG. 8. Tracheids and ray, with apparently the beginning of tylosis formation. × 375.

FIG. 9. Pitted tracheid from longitudinal section. × 375.

FIG. 10. Hyphae of fungus, lying in this instance between epidermis and ramenta; longitudinal section. × 280.

FIG. 11. Internal periderm and three gum canals which show typical schizogenous formation. × 60.

FIG. 12. Ramenta lying directly upon epidermis; no periderm is present in this region. Tranverse section. × 120.

PLATE XLIX

FIG. 13. Bundle from middle region of leaf-base; transverse section, showing phloem above, secondary xylem making up greater part of bundle, and primary xylem below. × 90.

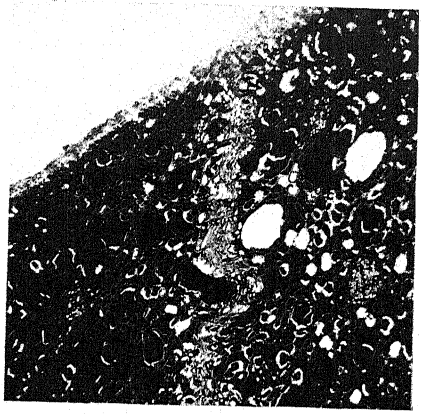
FIG. 14. Bundle from stalk of megasporophyll of *Cycas circinalis*, introduced for comparison. The secondary xylem is seen flanked on the outer side by secondary phloem, and on the inner side by primary xylem most of which is centripetal. × 80.

FIG. 15. Dorsal bundle from upper region of leaf-base, showing unusually well preserved phloem, also primary xylem. × 90.

FIG. 16. Detail of secondary xylem in transverse section, showing tracheids and the numerous rays with cells filled with decomposition product. × 225.

FIG. 17. Outer region of leaf-base, transverse section. Above are a number of ramenta, lying against the epidermis with its cuticle; below this is the periderm, followed by parenchyma, the cells as usual filled with dark-brown decomposition product. × 125.

FIG. 18. Stoma with guard cells and accessory guard cells, from longitudinal section through leaf-base. At the left are ramenta, and at the right the fundamental parenchyma. × 210.



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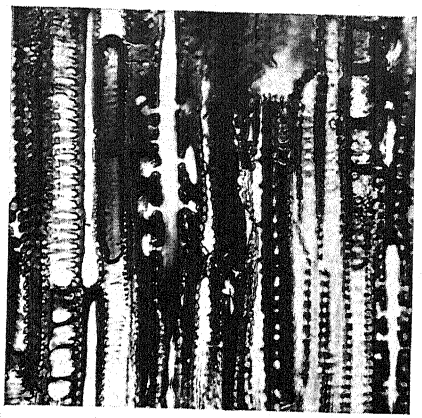

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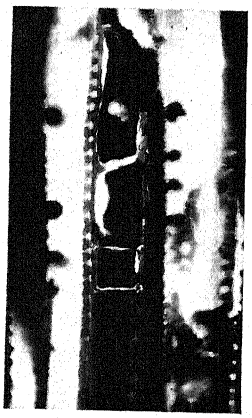

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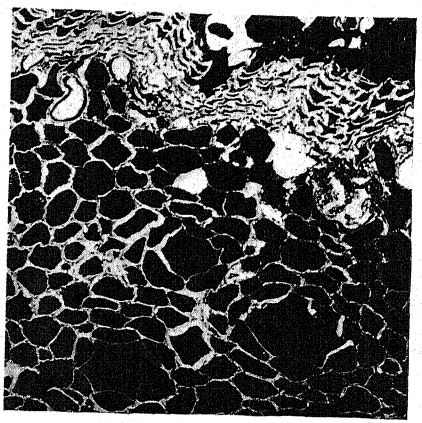

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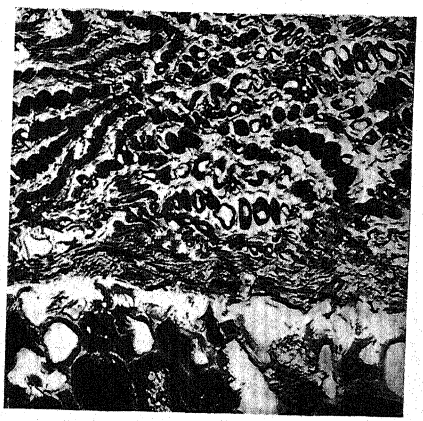

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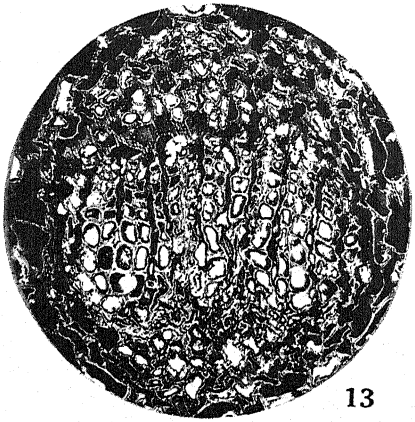

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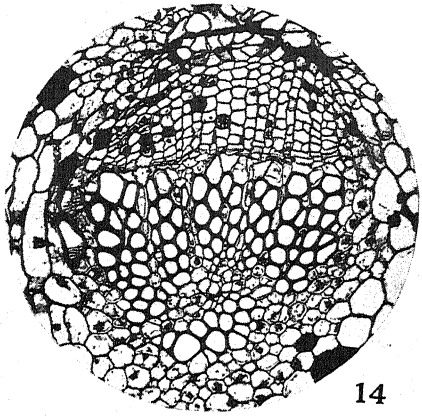

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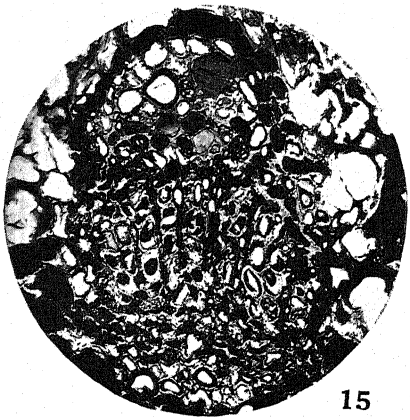

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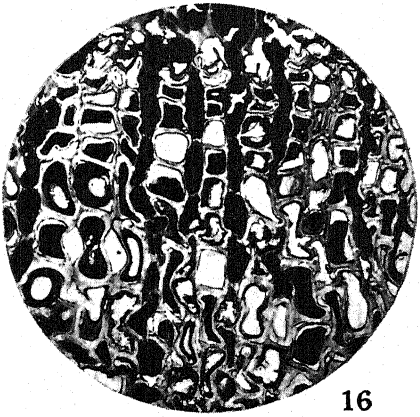

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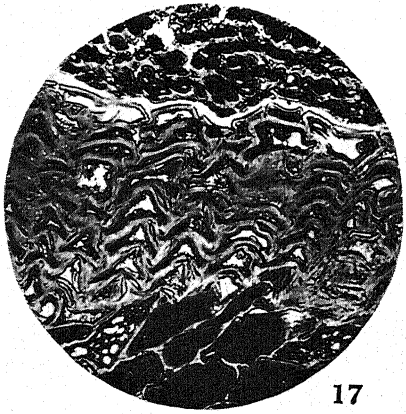

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THE MOVEMENT OF WATER THROUGH SOME SUBMERGED PLANTS [1]

HIRAM F. THUT

(Received for publication March 1, 1932)

Numerous data are available regarding the amount of water moving through land plants, but very little is known concerning the movement of water through submerged plants.

The current of water moving through the water-conducting system of a plant, regardless of the force or forces causing the movement, has been termed the "transpiration stream." Using the term in this broad sense, rooted submerged hydrophytes as well as ordinary land plants have a "transpiration stream." In land plants this stream is associated primarily with the evaporation of water from the leaves. If other forces are involved, it must be admitted that little is known about their relative significance. In submerged plants under normal conditions the factor of evaporation from the leaves is absent. In these plants, then, it is possible to measure the amount of the "transpiration stream" in the absence of evaporation.

The object of the present study was (1) to obtain data as to the amount of water moving through submerged plants and (2) to locate, if not determine, the force or forces active in causing the water movement.

HISTORICAL

The first actual demonstration of water movement in submerged plants was probably carried out by Unger (1862). He thought it unreasonable that leaves of amphibious plants when exposed to air should act as organs of transpiration and when suddenly submerged as organs of absorption. He postulated an upward current in water plants as well as in land plants and set up a demonstration experiment. Two vessels nearly full of water were placed side by side. The roots of a *Potamogeton crispus* plant were immersed in one vessel and the stem end bearing leaves was immersed in the second vessel. The central exposed portion of the stem was passed through a U-tube to prevent desiccation. At the end of eight days the weight of the water in the vessel containing the stem had increased 1.6 grams. By a similar set-up he found that the vessel containing the stem end of *Ranunculus fluitans* had increased .8 gram in eight days.

According to Snell (1908), Wieler (1892) attempted to verify Unger's results by "bleeding" experiments on *Elodea* and *Ceratophyllum*. He con-

[1] Papers from the Department of Botany, The Ohio State University, No. 298.

cluded that Unger was correct. However, Wieler's experiments were questioned by contemporary workers because of his inconsistent results.

Sauvageau (1891), according to Arber (1920), by sealing the cut end of stems of submerged plants with cocoa butter and making certain no roots were present on the stems, found that even under these circumstances the shoots thrived and developed new buds. His results were in agreement with the current view that water is absorbed through the leaves and stem. However, he concluded from a second experiment, in which a potometer method was used, that under normal conditions a definite upward current of water exists in submerged plants. This second experiment has been criticised by various workers because the arrangement of the potometer might have forced water through the plant.

The reduced and poorly developed water-conducting system of submerged plants led many early workers to conclude that roots of water plants are merely holdfasts and that the plant obtains its water supply by absorption through the total leaf and stem surface. Those holding this view were Schenck (1886), Sachs (1887), Strasburger and others (1903), and Hanning (1912).

The work of Unger was not accepted by Hochreutiner (1896) because he failed to show that the method of measurement was sufficiently accurate. Workers since then, including Hochreutiner, have concluded, from data obtained by direct or indirect experimental methods, that a "transpiration stream" does exist in some submerged plants. Hochreutiner devised the following experiment: Two vessels were set adjacent to each other. The first contained an aqueous solution of eosin and the second contained pure water. Two cut stems of *Ranunculus aquatilis* were so placed that the first stem had 1.5 cm. of its base immersed in the eosin and the tip in water, and the second stem had 9.5 cm. of its tip in eosin and the base in water. After one and one-half days, eosin had moved into the first stem a distance of 9.5 cm. The second stem showed a coloration in only 3 cm. of the vascular system near the stem tip. Similar results were obtained with several species of *Potamogeton*. From these results Hochreutiner concluded that there is an upward current of water in submerged plants.

Some experiments similar in principle to those of Hochreutiner but more striking in results were performed by Thoday and Sykes (1909). By using cotton plugs, they fastened bulbs containing aqueous solutions of eosin to the cut ends of submerged branches of *Potamogeton lucens*. After a definite interval of time they dissected the stem and examined it for eosin. Rates of from 5.7 to 9.5 cm. per minute were recorded. They concluded that there was a rapid water current in detached rootless stems of *P. lucens* and that this current was largely due to forces within the leaves.

Pond (1903) and Snell (1908) carried out many indirect experiments on the same subject. They allowed some plants to become rooted in a substratum and prevented others from rooting. Those plants that were allowed to root grew much better than those in which rooting was prevented. They

both concluded that the roots serve not only as holdfasts but also as absorbing organs for mineral salts. Snell stated further that under normal conditions there was an ascending stream of water which carried the mineral salts from the roots to the growing parts of the plants. His evidence for a "transpiration stream" in submerged plants is based on the assumption that such a stream is necessary for the transfer of mineral salts from the roots to the stem and leaves.

Pond made one direct measurement of water movement through a *Ranunculus aquatilis trichophyllus* plant. He sealed the root tip of the plant in a bottle by using a split rubber stopper. The amount of water absorbed by the root was measured by an indicator tube which was also inserted into the stopper of the bottle. The root used was 14 cm. long and covered with root hairs. It absorbed 5 cc. of water in 24 hours. Readings were taken on two succeeding days and only this one set-up was made. This measurement by Pond and the two measurements by Unger represent the available quantitative data on the amount of water movement through submerged plants.

Methods

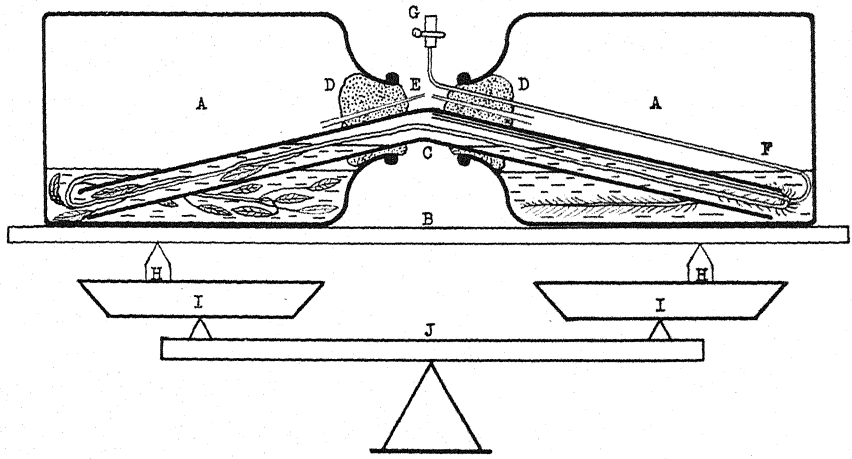
A number of different methods were devised in attempting to secure quantitative data concerning the amount of water moving through submerged plants. A weight method and a volume method proved successful. These two methods gave consistent results and are described below.

Measurement by Weight

A diagram of the apparatus that was devised for the weight method of measurement is shown in text figure 1. Two large-mouthed bottles (*A*) of two-liter capacity each were mounted, mouth toward mouth, several centimeters apart on a board (*B*). The two bottles were connected by means of a large, bent-glass tube (*C*). A plant was placed in the tube so that the roots of the plant were in one bottle and the stem tip was in the other. Sufficient water was added to each bottle to cover the ends of the glass connecting tube and the ends of the plant. The bottles were then stoppered with modeling clay (*D*). This clay ("Plasteline") is an oil-clay combination which is practically impermeable to water. A small capillary-tipped glass tube (*E*) was inserted through each stopper to permit equalization of pressure. The amount of water loss through such a tube and seal was found to be less than experimental error. A glass U-tube (*F*) of small bore was inserted into the apparatus before it was finally assembled so that one arm of the small tube extended up to the bend within an arm of the large tube (*C*) and the second arm of the small glass tube extended through the clay stopper. This second arm was closed by using a short piece of rubber tubing and a pinch cock (*G*). By means of this tube the level of the water in the large glass tube connecting the two bottles could be adjusted. Changes of sunlight and temperature

caused bubbles to accumulate in the bend of the large tube. The air space in the bend of the tube thus gradually increased. The water level was adjusted frequently to prevent having too much of the plant exposed to the air.

The whole apparatus was supported by two knife-blade-edged blocks of wood (*H*) setting in the pans (*I*) of a torsion balance (*J*). One bottle of the apparatus was thus supported over each pan. If the balance was tipped from side to side the blocks of wood acted as fulcrums. The apparatus was set with one pan of the balance slightly heavier than the other. Sufficient weights were then added to the second pan to tip the balance to that side. Only a slight tipping of the balance was used so that the water in the bottles remained practically undisturbed. Since the water in the two bottles was



TEXT FIG. 1. Diagram of weight method used in measuring the amount of flow of water through a submerged plant: *A*, large mouth bottle; *B*, board; *C*, large, bent, glass connecting-tube; *D*, modeling clay; *E*, capillary tipped glass tube; *F*, glass U-tube of small bore; *G*, rubber tubing and pinch cock; *H*, knife-blade-edged block of wood; *I*, pan of balance; *J*, torsion balance.

separated by an air space in the center of the large glass tube, the transfer of water from one side to the other through the plant would cause a difference in the amount of weights required to tip the balance. This weight difference was used in calculating the amount of water transferred. Before each weighing the size of the air space in the large glass tube was adjusted to the original weighing size. The tipping of the balance also had to be adjusted to the original conditions. These factors were carefully checked as they affected the ease with which the balance was tipped. Weighings were accurate to .2 of a gram.

The weight method experiments were performed in the greenhouse of the Botany Department of the Ohio State University. The experimental plants were grown in the greenhouse tanks. In transferring the plant from the

tanks to the apparatus great care was taken to prevent long exposure of the plant to the air. The roots were wrapped in cheesecloth to prevent the destruction of small roots and root hairs. The apparatus and the contained plant were subject to the temperature and daily light fluctuations of the greenhouse. The temperature usually varied from 18–32° C. The plant part contained in the bend of the large glass tube was shielded from direct sunlight to prevent possible detrimental effects of the sunlight on the part exposed to the air.

At the end of the experiment the differences of weight required to tip the apparatus from time to time were calibrated in terms of water volume by the following method: A known volume of water was inserted into the first bottle and the difference of weight required to tip the apparatus noted. An equal volume of water was then added to the second bottle and again the weight difference noted. By proportion, from the data thus obtained, the amount of water transferred by the plant was calculated.

The results of one set-up are given below. An *Elodea densa* plant with a stem length of 60 cm. was used. The apparatus was set up November 13, 1928.

TABLE 1. *Results Obtained by the Weight Method, from One Set-up of the Apparatus*

Date	Temperature in Room at Time of Weighing	Grams Required to Tip Balance to Root Side
Nov. 13, 8 p.m.	19° C.	122.8 g.
Nov. 13, 5 p.m.	17° C.	124.5 g.
Nov. 22, 8:30 a.m.	19° C.	125.4 g.
Nov. 28, 10:30 a.m.	23° C.	126.8 g.
Dec. 5, 10:30 a.m.	20° C.	128.3 g.
Experiment discontinued December 5, 1928.		

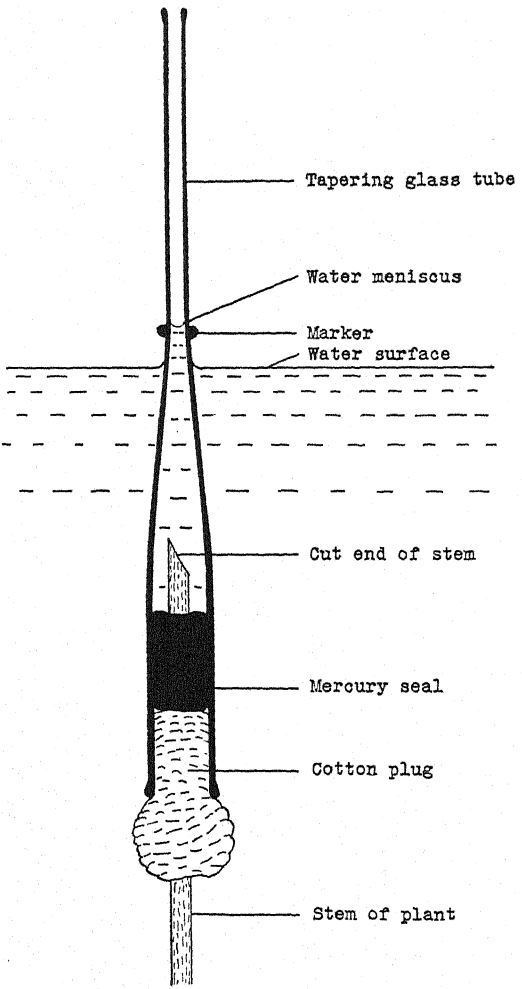
Ten cc. of water at 20° C. added to stem side required 13.3 grams on root side to balance and 10 cc. of water added to root side required 12.7 grams on stem side to balance. Ten cc. of water were thus equivalent to 13 grams of weight in this system. The difference in weight between November 13 and December 5 was 5.5 grams. Since 13 grams of weight equal 10 cc. of water in this system then 5.5 grams equal 4.23 cc. of water.

As the plant removed the water from one side and added it to the other the actual amount of water transferred was but one-half of 4.23 cc. or 2.115 cc. The 2.115 cc. gives an average hourly rate of .00408 cc. of water for the 518.5 hours of the experiment.

Several modifications of the above type of apparatus were devised and tried. Attempts were made to eliminate the large bottles and the relative inaccuracy in weighings due to their size. But when smaller bottles or only large tubes were used the plants usually died. This was probably due to insufficient aeration.

Measurement by Volume

The type of apparatus devised and used as the volume method is illustrated in text figure 2. In preparation for a set-up of the apparatus the plant to be used was cut off just below the water surface. Since the upper portion of the stems of many of the water plants either approach a horizontal position or



TEXT FIG. 2. Volume method of measuring flow of water from cut stem of submerged plant.

grow in a horizontal position just under the water surface, it was always possible to remove several centimeters of the stem tip and still have the cut end of the attached stem very close to the water surface. The rooted basal part of the plant stem was then wrapped with cotton, at a point approximately

one and one-half centimeters from the cut end, and a glass tube gently pushed over the cotton so that the cotton formed a plug and the cut end of the stem was contained within the glass tube. By the use of a pipette, mercury was inserted into the tube above the cotton plug so that a seal was formed about the stem when the glass tube was held in a vertical position.

The tube used was tapering and had an inside diameter of 8 mm. at the large end and of 2 mm. at the small end. This tube was supported with the large end containing the cut stem submerged and the small end of the tube extended above the water surface. A rubber band marker was adjusted on the small end of the tube to a height above the water surface equivalent to the height at which capillarity would support a water column in a tube of that size. The tube was then filled with water up to the marker. With this arrangement and this size tube volume changes of .01 cc. could easily be observed. Any increases in the water volume were removed and measured. Decreases of water volume were measured by the additions of water necessary to bring the water level up to the marker.

With this type of apparatus only a small part of the plant was contained within the glass tube. The shape of the glass tube allowed ready escape of gas bubbles from the cut stem. The accuracy of the method was but slightly affected by the changes of bubble rate from the plant due to environmental fluctuations. The effect of temperature changes of 10–15° C. on the water volume in such a tube was not noticeable. The actual amount of water contained in the several tubes used varied from .3–1. cc.

The volume method experiments were performed with the plants in the habitat in which they were growing. Some of the plants used in these experiments grew in the tanks of the greenhouse of the Botany Department of the Ohio State University. Several species of plants grew in a small lake (Baumgartner's Lake) located about eight miles south of Columbus, Ohio. A few of the plants used grew in Buckeye Lake, located about 30 miles east of Columbus, Ohio. The plants used in these experiments were subject to the temperature and light fluctuations of the habitat.

Table 2 contains the data obtained from eight stems of *Potamogeton*

TABLE 2. *The Amount of Water Flow from Potamogeton lucens Stems as Measured by the Volume Method*

Plant No.	Length of Stem Tip Cut Off (in cm.)	Set up A.M. July 1	Flow from Cut Stems Measured in cc. Readings Made at Following hours:						Total per Plant
			11 a.m.	1:30 p.m.	4 p.m.	6:30 p.m.	10 p.m.	July 2 8 a.m.	
1	12	8:20	.02	.03	.03	.015	.02	0	.115
2	13	8:20	0	0	0	0	0	0	0
3	9	8:30	.02	.015	.01	.004	0	0	.049
4	8	8:35	.04	.043	.04	.006	0	0	.129
5	9	9:00	.015	.01	.02	0	0	0	.054
6	5	9:05	.015	.032	.023	.005	0	0	.075
7	13	9:00	.021	.02	.025	.025	.015	0	.105
8	9	9:15	.01	.015	.02	.005	0	0	.05

lucens by the volume method. The experiment was run on July 1 and 2, 1929. The days were cloudy and only slight temperature fluctuations (23–24° C.) occurred in the water of the greenhouse tanks where the experiment was conducted. The stems of the plants were approximately 63 cm. long after the stem tips had been removed. Considerable individual variation will be noted in the amount of flow from the several plants tested. These data are representative of the data obtained by the volume method.

From table 2 and other similar data it was found that the flow from the cut stems gradually decreases and in 16 to 24 hours the flow practically ceases. The rate of flow for the first eight hours, however, remained nearly constant. For this reason a period of eight hours was arbitrarily chosen as the time interval for further volume measurements.

Other volume methods of measurement were tried. Attempts were made to include the whole stem tip and leaves in a glass tube. By such a method measurements could have been made on intact plants. The method, however, proved unsuccessful. Due to temperature and especially light fluctuations, bubbles of gas accumulated on the sides of the tube and on the stem and leaves. These bubbles caused great fluctuations in the volume of water and made accurate quantitative measurements impossible.

Results

The results obtained by using the weight method are given in tables 3 and 4. The averages for the results obtained by the volume method are given in table 5.

Table 3 gives the amount of water calculated to have passed through plants of *Elodea densa*. This tropical species of *Elodea* was used as it is

Table 3. *Water Movement through Elodea densa Plants as Measured by the Weight Method*

Plant No.	Date of Experiment	Length of Exp. (hrs.)	Temp. at Time of Weighings	Amt. of Water Transferred (cc.)	Rate per hr. Water Transferred (cc.)
1	Nov. 14–Dec. 5, 1928	453.0	17–23° C.	2.6	.0057
2	Nov. 13–Dec. 5, 1928	518.5	17–23° C.	3.46	.0067
3	Nov. 13–Dec. 5, 1928	518.5	17–23° C.	2.115	.0041
4	Dec. 22, 1928–Jan. 12, 1929	511.0	20° C.	9.81	.0192
5	Jan. 19–26, 1929	167.0	18–22° C.	1.22	.0073
				Average rate per hour =	.0086

larger and is easier to handle than the smaller native species. The plants grew well in the tanks of the greenhouse and there were but slight seasonal variations in growth. The plants used were of nearly equal size and approximately 65 cm. in stem length. The mean temperature during these experiments was 20° C. The data have been calculated on a volume basis at 20° C. Considerable variation occurred in the amount of water movement

through the five plants tested. The average amount was .0086 cc. per plant per hour.

Table 4 gives the data for *Potamogeton lucens* as obtained by the weight method. This species also grew well in the tanks of the greenhouse. The

TABLE 4. *Water Movement through Potamogeton lucens Plants as Measured by the Weight Method*

Plant No.	Date of Experiment	Length of Exp. (hrs.)	Temp. at Time of Weighings	Amt. of Water Transferred (cc.)	Rate per hr. Water Transferred (cc.)
1–2	Jan. 16–26, 1929 Av. for two plants	234	19–22° C.	5.337	.0228
3	July 15–21, 1930	142	24–31° C.	.667	.0047
4	July 15–21, 1930	142	24–31° C.	1.250	.0089
5	July 21–26, 1930	120	30–31° C.	1.444	.0120
6	July 21–26, 1930	120	30–31° C.	1.120	.0093
7	July 21–26, 1930	120	30–31° C.	1.037	.0086
				Average rate per hour =	.01105

plants used were approximately 65 cm. in stem length. The mean temperature was 25° C. The data have been calculated on a volume basis at 20° C. These plants also show considerable variation in the amount of water transferred per plant. The average for the seven plants tested was .011 cc. per plant per hour.

Table 5 gives the average flow for each of the several species of plants tested by the volume method. Comparison of the results from the weight and volume methods show that considerable similarity exists between the averages obtained by the two methods. *Elodea densa* plants, as determined by the weight method, showed an average of .0086 cc. of water transferred per plant per hour. Sixteen plants tested by the volume method in July, 1929, showed an average of .008 cc. of water transferred per plant per hour, and eight plants tested in October, 1929, showed an average of .0051 cc. transferred per plant per hour. These results are quite similar if one considers the variability of the individual plants and the conditions under which the several experiments were performed.

For *Potamogeton lucens* by the weight method the average rate of water transfer was .011 cc. per plant per hour. By the volume method 15 plants in June, 1929, averaged .0072 cc. per plant per hour and 10 plants in July, 1930, averaged .0111 cc. per plant per hour. Here again the results by the two methods are of approximately the same magnitude.

In comparing the "transpiration stream" of the several species of plants tested by the volume method the data of table 5 show that the strongly rooted *Potamogeton americanus* has the highest rate. Twelve plants averaged .0084 cc. per hour over a 24-hour period and five plants averaged .013 cc. per hour in an 8-hour period. *Potamogeton lucens* has a rate nearly equalling that of *P. americanus*. Rates of .0072 cc. and .0111 cc. per hour for 8-hour periods

TABLE 5. *Averages of the Amount of Flow from the Cut Stems of the Following Species of Plants as Measured by the Volume Method. Measurements Given are for an 8-hour Flow Period*

Species	Time	Temperature Limit	No. of Plants Tested	Stem-tip Cut Off. Length (cm.)	Length of Basal Portion of Stem	Amount of Water Flow	Rate of Flow in cc. per Hour
Elodea densa	July 21, 22, 1929	26–28.5° C.	16	16 cm.	63 cm.	.064 cc.	.008 cc.
do.	Oct. 14–15, 1929	19.5–20.5° C.	8	21	63	.041	.0051
Potamogeton lucens	June 25–July 2, 1929	23–26° C.	15	9	63	.0574	.0072
do.	July 9, 1930	25–27° C.	10	7	63	.089	.0111
Potamogeton americanus	Aug. 15–17, 1929	19.5–26° C.	12	16	65.3	.202*	.0084*
do.	May 5–9, 1930	21.5–24° C.	5	8	72	.104	.013
Ranunculus circinatus	May 5–9, 1930	21.5–24° C.	20	7.2	59	.0503	.0063
Myriophyllum spicatum	Aug. 13, 1929	26–27° C.	8	16.5	55.7	.021†	.0035†
Potamogeton compressus	June 26, 1929	26–29° C.	5	11	75	.021	.0026
Ceratophyllum demersum	June 26, 1929	26–29° C.	5	15.5	75	.002	.0003
do.	Aug. 2, 1930	29–31° C.	15	7.5	60	.001	.0001

* Measurements of flow for a 24-hour period.

† Measurements of flow for a 6-hour period.

have been recorded for two sets of plants. *Elodea densa* and *Ranunculus circinatus* have rates ranging between .008 cc. and .005 cc. per plant per hour for 8-hour periods. *Myriophyllum spicatum* and *Potamogeton compressus* have low rates with averages of .0035 cc. and .0026 cc. per plant per hour for 6- and 8-hour periods respectively. The rates for *Ceratophyllum demersum*, an unattached plant, are very low and come within the limits of experimental error.

Discussion

The results obtained in these experiments substantiate the much questioned results of Unger. A plant of *Potamogeton crispus* by his method transferred 1.6 grams of water through the plant in eight days. This gives an average hourly rate of .0083 gram. A plant of *Ranunculus fluitans* transferred .8 gram in eight days, which gives an average rate of .0042 gram per hour. Since grams and cubic centimeters of water are practically equivalent at ordinary room temperatures, Unger's results and those obtained in these experiments are seen to be quite similar.

The results obtained by Pond from his one set up of a *Ranunculus aquatilis trichophyllus* plant greatly exceeds the amount obtained in these experiments. The 5 cc. of water taken up by the root in 24 hours gives an average hourly rate of .208 cc. This rate is more than thirty times the hourly rate obtained as an average by the volume method for *Ranunculus circinatus* over an 8-hour period. This rate exceeds considerably the maximum rates obtained for any of the species of plants tested in these experiments. Table 6 gives the maximum hourly rate for single plants of each species as obtained by the volume

Table 6. *Maximum Rates for Single Plants of Each Species as Tested by the Volume Method over 8-hour Periods*

Species	Date	Hours	Temp. Limits	Water Flow (cc.)	Rate per Hour (cc.)
Potamogeton americanus	Aug. 16, 1929	8:30 a.m.–4:30 p.m.	19.5–22° C.	.295	.037
Elodea densa	July 21, 1930	8:30 a.m.–4:30 p.m.	26–28° C.	.19	.024
Potamogeton lucens	July 9, 1930	9-30 a.m.–5:30 p.m.	25–26° C.	.16	.02
Ranunculus circinatus	May 8, 1930	10:30 a.m.–7:00 p.m.	21.5–22° C.	.14	.016*
Potamogeton compressus	June 26, 1929	10:00 a.m.–6:00 p.m.	27–29° C.	.078	.01
Myriophyllum spicatum	Aug. 13, 1929	12:00 m.–6:00 p.m.	26–28° C.	.06	.01†
Ceratophyllum demersum	June 26, 1929	9:00 a.m.–5:00 p.m.	26–29° C.	.007	.001

* Rate per hour for an 8½-hour period.

† Rate per hour for a 6-hour period.

method over 8-hour periods. A plant of *Potamogeton americanus* has the high rate of .037 cc. per hour. The rate of .208 cc. per hour obtained by Pond exceeds this by nearly six times. The .208 cc. rate exceeds by thirteen times the maximum rate of .016 cc. obtained from a *Ranunculus circinatus* plant.

TABLE 5. *Averages of the Amount of Flow from the Cut Stems of the Following Species of Plants as Measured by the Volume Method. Measurements Given are for an 8-hour Flow Period*

Species	Time	Temperature Limit	No. of Plants Tested	Stem-tip Cut Off. Length (cm.)	Length of Basal Portion of Stem	Amount of Water Flow	Rate of Flow in cc. per Hour
Elodea densa	July 21, 22, 1929	26–28.5° C.	16	16 cm.	63 cm.	.064 cc.	.008 cc.
do.	Oct. 14–15, 1929	19.5–20.5° C.	8	21	63	.041	.0051
Potamogeton lucens	June 25–July 2, 1929	23–26° C.	15	9	63	.0574	.0072
do.	July 9, 1930	25–27° C.	10	7	63	.089	.0111
Potamogeton americanus	Aug. 15–17, 1929	19.5–26° C.	12	16	65.3	.202*	.0084*
do.	May 5–9, 1930	21.5–24° C.	5	8	72	.104	.013
Ranunculus circinatus	May 5–9, 1930	21.5–24° C.	20	7.2	59	.0503	.0063
Myriophyllum spicatum	Aug. 13, 1929	26–27° C.	8	16.5	55.7	.021†	.0035†
Potamogeton compressus	June 26, 1929	26–29° C.	5	11	75	.021	.0026
Ceratophyllum demersum	June 26, 1929	26–29° C.	5	15.5	75	.002	.0003
do.	Aug. 2, 1930	29–31° C.	15	7.5	60	.001	.0001

* Measurements of flow for a 24-hour period.

† Measurements of flow for a 6-hour period.

have been recorded for two sets of plants. *Elodea densa* and *Ranunculus circinatus* have rates ranging between .008 cc. and .005 cc. per plant per hour for 8-hour periods. *Myriophyllum spicatum* and *Potamogeton compressus* have low rates with averages of .0035 cc. and .0026 cc. per plant per hour for 6- and 8-hour periods respectively. The rates for *Ceratophyllum demersum*, an unattached plant, are very low and come within the limits of experimental error.

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Table 6. *Maximum Rates for Single Plants of Each Species as Tested by the Volume Method over 8-hour Periods*

Species	Date	Hours	Temp. Limits	Water Flow (cc.)	Rate per Hour (cc.)
Potamogeton americanus	Aug. 16, 1929	8:30 a.m.–4:30 p.m.	19.5–22° C.	.295	.037
Elodea densa	July 21, 1930	8:30 a.m.–4:30 p.m.	26–28° C.	.19	.024
Potamogeton lucens	July 9, 1930	9-30 a.m.–5:30 p.m.	25–26° C.	.16	.02
Ranunculus circinatus	May 8, 1930	10:30 a.m.–7:00 p.m.	21.5–22° C.	.14	.016*
Potamogeton compressus	June 26, 1929	10:00 a.m.–6:00 p.m.	27–29° C.	.078	.01
Myriophyllum spicatum	Aug. 13, 1929	12:00 m.–6:00 p.m.	26–28° C.	.06	.01†
Ceratophyllum demersum	June 26, 1929	9:00 a.m.–5:00 p.m.	26–29° C.	.007	.001

* Rate per hour for an 8½-hour period.

† Rate per hour for a 6-hour period.

method over 8-hour periods. A plant of *Potamogeton americanus* has the high rate of .037 cc. per hour. The rate of .208 cc. per hour obtained by Pond exceeds this by nearly six times. The .208 cc. rate exceeds by thirteen times the maximum rate of .016 cc. obtained from a *Ranunculus circinatus* plant.

The plant having the highest hourly rate of flow (table 6) exceeds from two to five times the average for that species (table 5). Rates of flow for individual plants thus range from two to five times that of the average flow to zero in those individuals that failed to show water movement.

Location of Water-moving Forces

For *Elodea densa* and *Potamogeton lucens* the amount of water flow from the cut surface of the basal part of the plant, as measured by the volume method, was for a time practically equivalent to the amount of water transferred by the complete plant, as measured by the weight method. If the forces of the basal part of the plant cause a water flow practically equivalent to that of the complete plant then the water-moving forces present in the upper stem and leaves must be slight. By the basal part of the plant is meant the lower two-thirds of the plant stem, with the roots and the attached leaves. The upper stem includes about one-third of the stem length of the plant, including the stem tip and the attached leaves.

Measurements were made on the amount of water taken up at the cut stem end by the upper stem and attached leaves. A slight modification of the volume method was used in making these measurements. The upper stem was first cut from the plant; cotton was wrapped around the stem at a point approximately 1½ cm. from the cut end, and then a glass tube was pushed over the cotton. Sufficient mercury was inserted within the glass tube so that the tube could be held at an angle from the perpendicular and still have a good seal. The tube was then fastened rigidly. By placing the tube obliquely the curving stem tip would assume a nearly natural position. The marker on the tube was adjusted at a height above the water surface equalling capillarity of water in that sized tube. The tube was then filled up to the marker with water. The amount of water taken in by the upper stem was measured by the amount of water required to bring the water line within the tube up to the marker. The amount of water taken up by the upper stem is given in table 7 for the four species of plants used in these experiments. The amount of flow from the basal part of the plant is also included in the table for these same species.

These data indicate that for *Potamogeton lucens* the amount of flow from the rooted basal portion of the plant was seven to eleven times greater than the amount of water entering the upper stem. For *Elodea densa* the flow from the basal part was ten to sixteen times greater than the water entering the upper stem. *Myriphyllum spicatum* showed a difference of twelve times, and *Potamogeton americanus* thirteen to twenty times, in the two water volumes.

Evidently the quantity of water moved in the stem base and roots exceeds from seven to twenty times the water entering the upper stem and leaves as measured by actual water volume data in the species of plants tested. The

TABLE 7. *The Amount of Water Flow from the Basal Part of the Stem and the Amount of Water Taken up at the Cut Surface of the Upper Stem. Results Obtained by the Volume Method. Average Results are Given*

Stem Part Used	Time	Temperature Limits	No. of Plants Tested	Length of Stem Cut Off	Length of Stem Base	Length of Exp. (hrs.)	Amount of Water Flow	Rate per Hour (cc.)
Potamogeton lucens								
Upper	July 8–10, 1929	25.5–27.5° C.	16	27.2 cm.	—	8	.009 cc.	.001 cc.
Base	June 25–July 2, 1929	23–26° C.	15	9	63 cm.	8	.0574	.0072
Base	July 9, 1930	25–27° C.	10	7	63	8	.089	.0111
Elodea densa								
Upper	July 11–16, 1929	24–27° C.	16	15	—	8	.004	.0005
Base	Oct. 14–15, 1929	19.5–20.5° C.	8	21	63	8	.041	.0051
Base	July 21–22, 1930	26–28.5° C.	16	16	63	8	.064	.008
Myriophyllum spicatum								
Upper	Aug. 13–14, 1929	23.5–25° C.	8	18.1	—	24	.0056	.0003
Base	Aug. 13, 1929	26–27.5° C.	8	16.5	55.7	6	.021	.0035
Potamogeton americanus								
Upper	Aug. 19–20, 1929	21.5–24° C.	11	23	—	8	.0052	.00065
Base	Aug. 15–17, 1929	19.5–26° C.	12	16	65.3	24	.202	.0084
Base	May 5–9, 1930	21.5–24° C.	5	8	72.0	8	.104	.013

water-moving forces of submerged plants thus seem to be principally located in the rooted basal part of the plant.

It might be objected that cutting the stem results in a clogging of the water-conducting tissues. An experiment was set up to determine whether water passed into the cut stem ends of the water plants. *Potamogeton americanus* has both submerged and floating leaves. Some upper stems were obtained that had only submerged leaves. Other upper stems had both submerged and floating leaves. The amount of water entering the two types of upper stems was measured and compared with the amount of water flow from the rooted basal portion of the plant. Table 8 contains the data obtained from the three different types of stems. The experiment was conducted at Baumgartner's Lake or in lake water on August 15–20, 1929. The results are averages for the plants tested.

TABLE 8. *The Amount of Water Flow from the Basal Stem; the Water Entering the Upper Stem with only Submerged Leaves; and the Water Entering the Upper Stem with Floating Leaves of Potamogeton americanus*

Stem Part Used	Temperature Limits	No. of Plants Tested	Lgth. of Stem Tip Cut Off or Used (cm.)	Lgth. Stem Base (cm.)	No. of Floating Leaves	Lgth. of Exp. (hrs.)	Amt. of Water (cc.)	Rate per Hr. (cc.)
Base	19.5–26° C.	12	16	65.3	——	24	.202	.0084
	21.5–24° C.	5	8	72.0	——	8	.104*	.013
Tip with submerged leaves only	21.5–24° C.	11	23	——	——	8	.0052	.00065
Tip with floating leaves	21–23° C.	9	21	——	4.6	8	.08	.01
	21.5–24° C.	5	5.4	——	4.6	8	.152	.019

* Data obtained May 5–9, 1930.

The upper stems having floating leaves, with rates of .01 cc. and .019 cc. per plant per hour, had a water intake exceeding the rates of .0084 cc. and .013 cc. per plant per hour flow from the basal parts of the plants. Water is taken in at the cut ends of the upper stems and at fairly high rates, especially if the water-moving forces in the stem and leaves are active.

Since the basal part of the plant used in the volume method included both the roots and two-thirds of the plant stem, the flow may be caused by forces located either in the roots or stem. Two experiments were set up in an attempt to more definitely locate the forces causing the flow. Submerged plant segments were obtained by cutting the stem just above the root or root stock and removing approximately 5 cm. of the stem tip. Attempts were made by the volume method to measure the movement of water through these middle stem segments. The results are given in table 9. The experiments were conducted August 16–19, 1930, in the greenhouse tanks. The temperature limits were 22–23° C. Average results are given.

TABLE 9. *The Amount of Water Movement through Middle Stem Segments as Measured by the Volume Method*

Species	No. of Stem Segments Used	Length of Stem Segments	No. of Leaves per Segment	Length of Experiment	Amount of Flow
Potamogeton americanus *	12	44 cm.	4.8	15 hrs.	0
Elodea densa	8	39	——	10	0
Potamogeton lucens	16	49.9	8.1	10	0

* Stem segments obtained from Baumgartner's Lake.

In a second experiment measurements were made on the short basal rooted part of the stem. The plant stem was cut a few centimeters from the roots or rootstock and the measurements of water flow made on this short stem portion. Measurements were made on a few *Potamogeton americanus* plants at Baumgartner's Lake. The surface of the lake was approximately three feet below the normal level and several plants that could be used for experimentation were found growing in shallow water. Plants of *Elodea densa* and *Potamogeton lucens* were propagated in the greenhouse tanks. These plants were rooted in pots of soil. After the plants had become well established the pots were raised and supported near the water surface. The water flow measurements made on these plants are given in table 10.

TABLE 10. *Measurements of the Flow of Water from the Cut Ends of the Short, Basal, Rooted Stems as Measured by the Volume Method. Average Results are Given*

Species	Temperature Limits	No. of Plants	Lgth. of Stem (cm.)	No. Hrs. of Flow	Amt. of Flow (cc.)	Rate per Plant per Hr. (cc.)
Potamogeton americanus	25–25.5° C.	4	7.5	8	.057*	.0071
Elodea densa	22–23° C.	9	9.3	8	.05*	.0062
Elodea densa	18–20° C.	6	7.0	8	.088†	.011
Potamogeton lucens	18–20° C.	10	8.5	8	.046†	.0057

* Measurements made Aug. 16–19, 1930.

† Measurements made Jan. 1, 1932.

Table 9 shows that the middle stem segments fail to cause water movement. If a gradient exists in the stem, which causes the movement of water upward in the plant, the force is quite negligible, especially if that part of the stem is cut from the stem tip and roots. The flow from the short basal stems, as recorded in table 10, is practically equivalent to the rate of flow through the complete plant. The flow of water through the submerged plants studied thus seems to be due primarily to "root pressure."

The very low results obtained in these experiments for *Ceratophyllum* and the inconsistent results obtained by Wieler in his "bleeding" experiments on *Ceratophyllum* and *Elodea* are explainable if the water flow through submerged plants is caused primarily by "root pressure." Most *Ceratophyllum* plants have either a very poorly developed embryonic root system or no root

system at all. The basal part of the plant frequently dies and the upper stem continues more or less as a free-floating plant.

Summary

Little is known concerning the "transpiration stream" of submerged plants, and very few data from actual quantitative measurements have been available.

A weight method and a volume method of measurements were devised in this investigation whereby quantitative measurements were made on the flow of water through submerged plants. Measurements of flow through the complete plant, covering fairly long time intervals, could be made by the weight method. Accurate measurements over short time intervals could be made by the volume method, which was essentially a method measuring "bleeding."

An average rate of flow of water through the plant of .0086 cc. per plant per hour was obtained for five *Elodea densa* plants by the weight method.

An average rate of .011 cc. per plant per hour was obtained for seven *Potamogeton lucens* plants by the weight method.

Of the plant species tested by the volume method, *Potamogeton americanus* had the highest hourly average flow from the cut end of the basal portion of the plant. Rates of .0084 cc. and .013 cc. per plant per hour were obtained for two sets of plants. *P. lucens* with average rates of .0072 and .0111 cc. per plant per hour for two sets of plants has rates nearly equalling those of *P. americanus*. Rates of .0051 and .008 cc. per plant per hour were obtained for *Elodea densa*. An average flow of .0063 cc. per plant per hour was obtained for *Ranunculus circinatus*. *Myriophyllum spicatum* and *Potamogeton compressus* had lower rates with averages of .0035 cc. and .0026 cc. per plant per hour, respectively. The rate obtained for *Ceratophyllum demersum* plants was very low, and came within the limits of experimental error.

The results obtained by the weight and volume methods on *Elodea densa* and *Potamogeton lucens* were found to be comparable.

In comparing the amount of water flow from the basal part of the plant with the amount of water entering the upper stem and submerged leaves it was found that the former exceeded the latter from seven to twenty times. These measurements were made by the volume method. The "transpiration stream" of the plants studied thus appears to be caused primarily by the forces in the basal part of the plant. The water-moving forces of the upper stem and submerged leaves appear to have but slight effect on the flow.

Middle stem segments did not cause water movement.

The flow from short-rooted, basal parts of the plant is practically equivalent to the rate of flow through the complete plant. The flow of water through the submerged plants studied thus seems to be due primarily to "root pressure."

Unger's two quantitative determinations of water flow through submerged plants were substantiated in these experiments. The one quantitative measurement of water through a submerged plant made by Pond was found to be much higher than those obtained in these experiments. The very low results obtained in these experiments for *Ceratophyllum* and the inconsistent results obtained by Wieler in his experiments are explainable if the water flow through submerged plants is caused primarily by "root pressure." *Ceratophyllum* plants have either a very poorly developed root system or no root system at all.

The writer wishes to express his indebtedness to Dr. E. N. Transeau, under whose direction this study was made, Dr. H. C. Sampson, Dr. L. H. Tiffany, and Dr. B. S. Meyer for suggestions and encouragement during the progress of this investigation and for valuable criticism of the completed manuscript.

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INVESTIGATIONS ON THE MORPHOLOGY AND COMPARATIVE HISTORY OF DEVELOPMENT OF FOLIAR ORGANS IV. THE PROPHYLL OF *CARYA BUCKLEYI* VAR. *ARKANSANA* [1]

ADRIANCE S. FOSTER

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INTRODUCTION

In *Carya Buckleyi* var. *arkansana* Sarg., the outermost scale of axillary buds on normal spur or long shoots is sac-like in form and, except for a more or less prominent opening near the abaxial apex, completely embraces the inner organs of the bud (text figs. 1, 4, 9). Since the axillary buds of many dicotyledons are provided with paired lateral prophylls [2] this sac-like prophyll of the black hickory is unusual and invites comparison with the characteristically addorsed prophyll of monocotyledonous buds.[3]

Apparently the earliest morphological interpretation of the prophyll of *Carya* was proposed by Döll (1848, p. 22 and fig. 18). He suggested that in *Carya alba,* the outer scale of the axillary bud represents two "inwardly fused prophylls." Later, Casimir de Candolle (1862, pp. 10–12 and Pl. I) in his memoir on the Juglandaceae, pointed out differences in the number, arrangement and character of the bud scales of *Carya* by the use of which he subsequently (1864, pp. 142–145) divided the genus into two sections. De Candolle emphasized the fact that members of the section Apocarya differ in the construction of their axillary buds as follows. In *Carya amara* (= *C. cordiformis* K. Koch) the buds possess paired lateral valvate prophylls. In *Carya aquatica* and *Carya olivaeformis* (= *C. pecan* Asch. and Gr.), however, de Candolle stated that the prophylls of lateral buds are fused together, forming a sheath.[4] Apparently unaware of Döll's (op. cit.) earlier work, de Candolle, failed to comment on the prophylls of members of the section Eucarya. However, the bud diagram given of *Carya porcina* (= C. *glabra* Sweet) clearly shows that in this species, the outermost scale of the axillary bud has a tubular character.[5]

[1] Contribution from the Botanical Laboratory of the University of Oklahoma, N. S. No. 17.

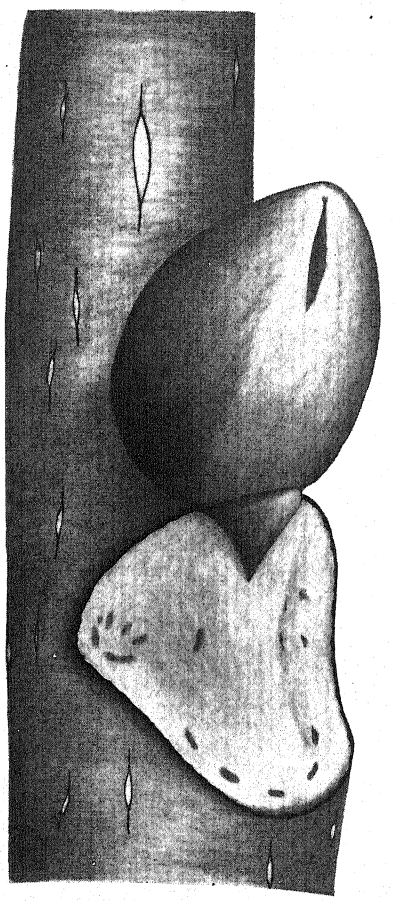
[2] Cf. Wydler (1843), Henry (1846), Goebel (1905, pp. 382–383) and Velenovský (1907, pp. 675–677).

[3] Cf. Rüter (1918) and Arber (1925, pp. 131–135).

[4] In his monograph in the Prodromus (p. 144) the description reads "gemmis flavis squamis 2 exterioribus lateralibus basi in tubum connatis."

[5] Cf. also Schneider (1903, p. 145 and fig. 88).

As far as I am aware, no effort has been made in recent years to test the validity of either Döll's or de Candolle's interpretation of the prophyll in *Carya*. Engler (1894, p. 20) for example, merely states that in *Carya* "the two outermost cataphylls are fused together." Even Trelease (1896, p. 36) in his detailed study of American Juglandaceae, simply describes the outermost



TEXT FIG. 1. Abaxial-lateral view of an adult axillary bud, November 24, 1929. Note the small elliptical opening near the apex of the sac-like prophyll. At the base of the axillary bud is a small accessory serial bud. The numerous colleters occurring on the buds have been omitted from the drawing (× 12).

scale on lateral buds of members of the section Eucarya as "a closed sac soon splitting from the top." The need for a more careful study of the question of prophyll morphology in *Carya* was thus apparent, especially in view of the systematic value of bud characters in this genus.

The present paper deals largely with the morphology of the prophyll in *C. Buckleyi* var. *arkansana*. Evidence obtained from many sources will be

presented in favour of the idea that in this species the prophyll is clearly bifoliar in organization. It will be further shown that in all probability this interpretation can be applied to the prophylls of a number of other species of the genus, although the desirability of more extensive comparative studies is definitely emphasized.

Form and Position of the Prophyll

The bicarinate character of the prophylls of many monocotyledons and of certain dicotyledons, e.g. *Salix* [6] and *Viburnum Opulus* [7] has repeatedly been cited as evidence of their bifoliar nature. From this standpoint, the form of the prophyll in *Carya* is of interest since it has been described by Cook (1916, p. 542) as being "a closed sheath, carinate on each side, somewhat similar to the prophylla of grasses and palms."

In the black hickory, the prophyll on buds in the mid region of normal spur or long shoots is slightly bicarinate near its apex (text fig. 1) but in median section view (text fig. 9) it will be seen that the keeled character has disappeared. The upper axillary buds of long shoots (cf. Foster, 1931, Pl. LXIII, fig. 1), however, are more flattened in form and their prophylls are definitely bicarinate above the middle. Indeed, transverse sections above the abaxial opening of the prophyll of such buds (text figs. 2–4) reveal a form quite analogous to certain monocotyledonous prophylls.[8] The form of the prophyll on buds of coppice shoots is significant. Not only is the bicarinate form of the prophyll well-marked in such buds, but likewise the abaxial edges of the prophyll may be free nearly or quite to the base of the bud. In one shoot examined, the adaxial region of the prophyll, which normally is only slightly notched (text fig. 1) was so deeply cleft that careful inspection was necessary to avoid the impression of paired, dorsally keeled prophylls. During the period of bud swelling and expansion in the spring, the prophyll of buds on normal and coppice shoots usually splits to the base on both its abaxial and adaxial faces. As a result, two discrete asymmetrical scales are formed, the dorsal keels of which simulate midribs of two independent foliar organs.

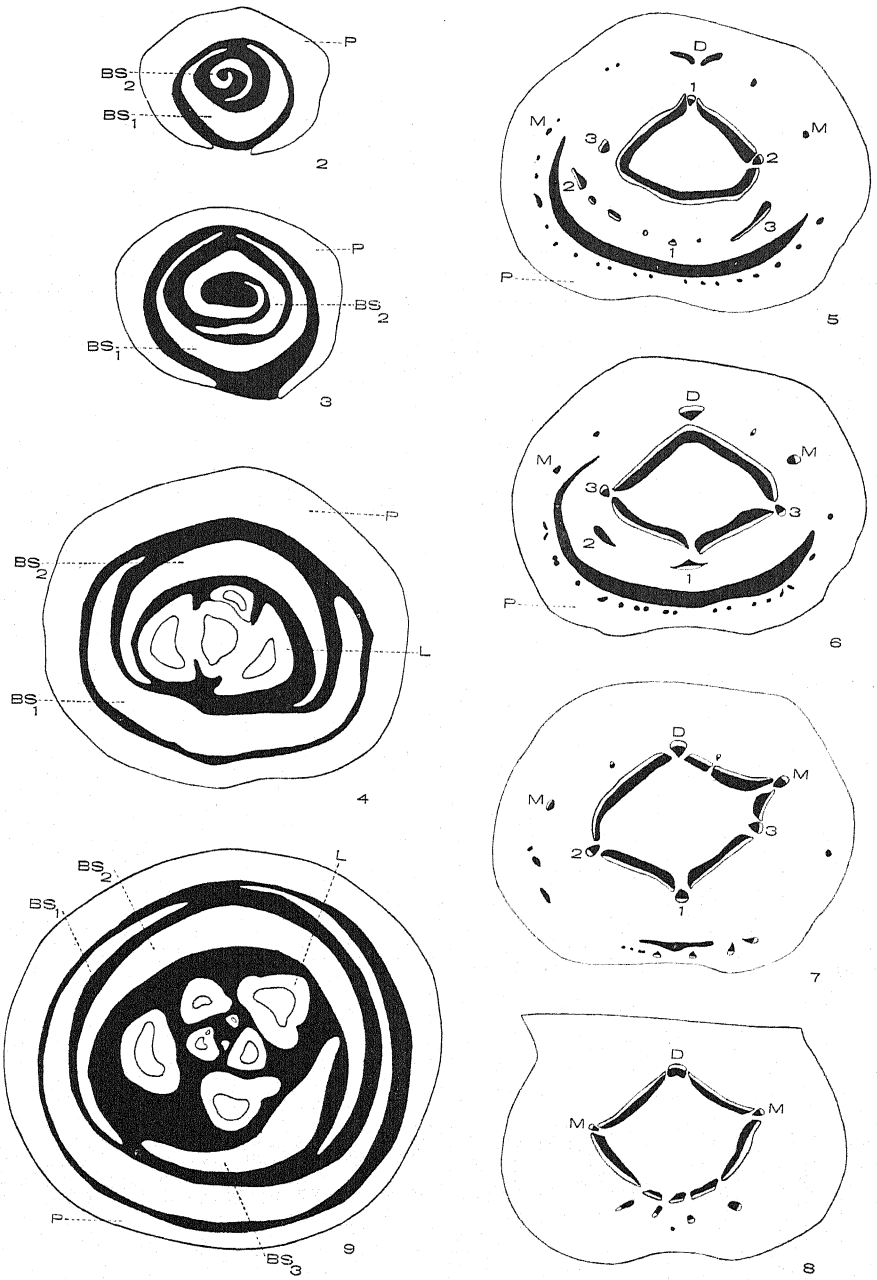
If, as the evidence from external form suggests, the prophyll represents two connate leaves, its position in relation to the other organs of the bud demands explanation. Text figures 4 and 9 represent two typical conditions found in buds of normal spur or long shoots. In both figures it will be seen that the prophyll is followed by two "distichous" median scales, a situation which appears invariable from my observations. In text figure 4, the second bud scale is followed directly by the spirally-arranged foliage leaves [9] while in text figure 9, the spiral phyllotaxy is initiated by a third scale. In general,

[6] Cf. Lubbock (1908, pp. 110–111, fig. 176) and Resvoll (1909, p. 332).

[7] Cf. Lubbock (1908, p. 117, pl. II, fig. 1).

[8] Cf. Arber (1925, fig. CIV) and Rüter (1918).

[9] The fractional divergence is approximately 2/5, as I have already pointed out in connection with the terminal bud (Foster, 1931, text. fig. 1 and p. 869).



TEXT FIGS. 2–9. Figures 2–8 represent transverse sections of an adult axillary bud at successive levels from apex to base. Bottom portion of all figures corresponds to abaxial surface of bud. Figs. 2–3, through the abaxial opening of the bicarinate prophyll (× 14). FIG. 4, median section showing prophyll surrounding the two distichous scales and three spirally arranged foliage leaves (× 12). FIGS. 5–8, successively lower sections showing nodal anatomy of scales and prophyll (× 12). FIG. 9, median transverse section through a larger axillary bud with three scales, the third initiating the spiral arrangement of succeeding foliar organs (× 15). In all figures: *P*, prophyll; *BS*, scale; *L*, foliage leaf. Phloem of stele and larger bundles white; xylem black. Smaller bundles shown in solid black. Spaces between foliar organs in the bud shown in black.

there is a progressive decrease in the number of scales of successive buds on a given shoot, those near the base having as many as five or six while in the uppermost buds the number is reduced to two usually. In all buds, however, the spiral phyllotaxy is initiated by the third organ following the prophyll, regardless of whether it may be a cataphyll or a foliage leaf. The unusual position of the first two scales could be explained by assuming that they represent a pair of foliar organs descussate with the two laterally connate phyllomes which have formed the prophyll. Such an hypothesis is supported by the fact that in buds of extremely vigorous coppice shoots, the prophyll is followed by a number of decussate pairs of scales (as many as three) before the 2/5 divergence of the inner foliar organs is initiated.[10] Further discussion of the significance of the arrangement of bud organs to the morphological interpretation of the prophyll will appear when the ontogenetic evidence is presented.

Nodal Anatomy

As I have previously shown (Foster, 1931, text fig. 1 and p. 876), the cataphylls and foliage leaves of the terminal bud possess a trilacunar node, a situation agreeing with the fundamental nodal anatomy of the Juglandaceae (Sinnott, 1914, p. 310). In striking contrast, the prophyll is typically multilacunar, a condition which might be expected in the case of a bifoliar organ.[11] The number of traces supplying the prophyll is quite variable, not only in buds from different shoots but also in the successive buds of the same shoot. In the fourteen buds which were studied, the vascular supply of the prophyll ranged from five to eleven separate traces from as many gaps. The relative frequency of each condition would be of little significance in view of the few buds examined but it may be of value to note that higher numbers (such as 9, 10, or 11 traces) seem more characteristic than the lower ones. As an example of the erratic variation in the nodal anatomy of the prophyll may be cited the case where in two successive buds of the same shoot, the prophylls were supplied, respectively, with six and eleven traces.

The nodal anatomy of the prophyll and scales in a typical case is shown in text figures 4–8. Near the base of this bud (text fig. 8), eight of the ten traces supplying the prophyll may be seen at varying distances from the stele. The five traces which have left the abaxial[12] region of the stele fork repeatedly and innervate the abaxial and a portion of the lateral regions of the prophyll (cf. text figs. 7, 6 and 5). The three traces leaving the adaxial region of the stele (text fig. 8) are of considerable interest. Two of them

[10] An analogous situation is described by Fankhauser (1882, p. 5 and pl. III, fig. 10) in the axillary buds of *Ginkgo biloba* L.

[11] The data presented here were obtained from a study of serial sections cut by hand, stained with phloroglucin and HCl and mounted in glycerine.

[12] The terms abaxial and adaxial are used here to describe, respectively, the portion of the bud stele away from or adjacent to the mother shoot.

(indicated by the letter *M*) prove to be strong unbranched strands which extend through the lateral keels of the prophyll; the other trace (labeled *D*) quickly forks after leaving the stele (text figs. 6 and 5) and, in part, innervates the adaxial region of the prophyll. The two remaining traces supplying the prophyll leave from the adaxial region of the stele, one at either side of trace *D*; they further contribute to the venation of the keeled region of the prophyll (text figs. 6 and 5). The two scales following the prophyll are trilacunar;[13] in text figures 7–5, the median traces of these scales are indicated by the number 1, the lateral traces by the numbers 2 and 3. Text figure 7 shows the departure from the stele of the three traces supplying the first scale. At successively higher levels (text figs. 6 and 5), the lateral traces are seen to converge in the scale base towards the repeatedly forking median bundle. The trilacunar node of the second scale is illustrated by text figure 5; the behavior of its median and lateral traces is similar to that just described for the first scale.

When the evidence from nodal anatomy is examined closely, the following highly significant fact emerges. Regardless of the exact number of traces supplying the prophyll, two of them (cf. traces *M* in text figs. 8–5) are constant in position and behavior. These traces, which innervate the lateral keels of the prophyll, logically seem to represent the median unbranched traces of two connate foliar organs. The other prophyllary traces tend to be variable in number and position in the stele; it would seem reasonable to interpret them as accessory traces which are related perhaps to the amplexicaul character of the prophyll.

Venation[14]

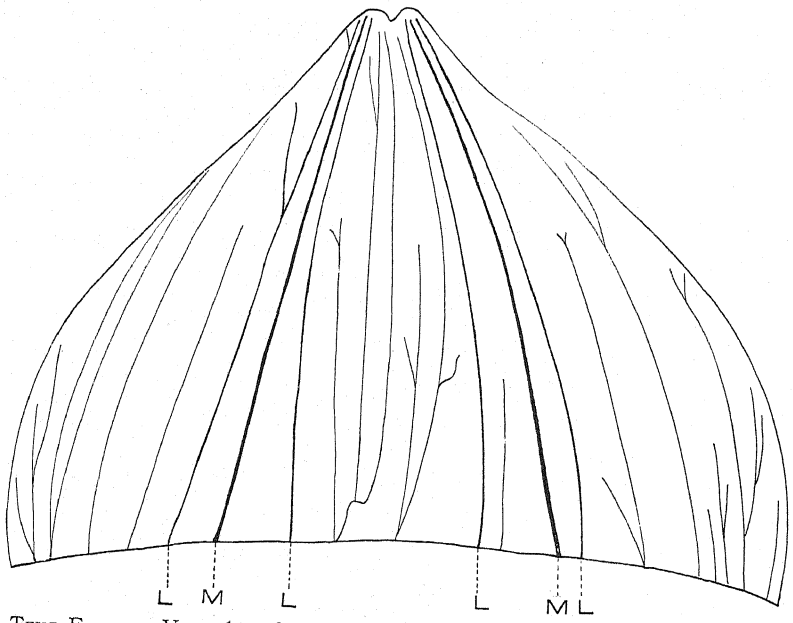
In correlation with its multilacunar node, the prophyll is traversed by a large but variable number of apically converging veins (text fig. 10). Although extensive variation occurs in the amount and character of the branching of the adaxial and abaxial veins (*i.e.* those which are derived from the accessory prophyllary traces) one significant feature of the vein system has been found to be constant, namely, the presence of a trio of strong veins in the region of each lateral keel. The mid veins of each trio (labeled *M* in the figure) are the strongest of all the prophyllary veins and are derived from the median prophyllary traces (cf. text fig. 8). These two veins, of equal strength, extend unbranched into apices of the prophyll where they are joined by the two converging laterals (labeled *L*). These lateral veins in each trio are derived, respectively, from the two small adaxial traces (*i.e.* those at either side of trace *D*) and from branches of the proximal abaxial traces (cf. text figs. 8–5). From the examination of twelve prophylls, considerable variation

[13] This is true of the scales of all of the axillary buds which have been studied.

[14] Prophylls were obtained from buds collected September 25, 1931, and cleared for venation studies by a method previously employed for the bud scales (Foster, 1931, p. 865).

appears to occur as regards the amount of branching of the lateral veins of each trio. In some cases, as is shown in the left trio of text figure 10, one of these lateral veins may give off several weak veins before its apex is reached while in other instances, both laterals may be unbranched like the median.

Each trio of strong veins in the prophyll suggests a separate and distinct "vein system." If this interpretation is sound, the lateral keeled portions



TEXT FIG. 10. Ventral surface of a flattened prophyll showing the complete venation, September 25, 1931 (× 14). *M*, strong unbranched median vein of each trio; *L*, lateral vein of each trio. Further explanation in the text.

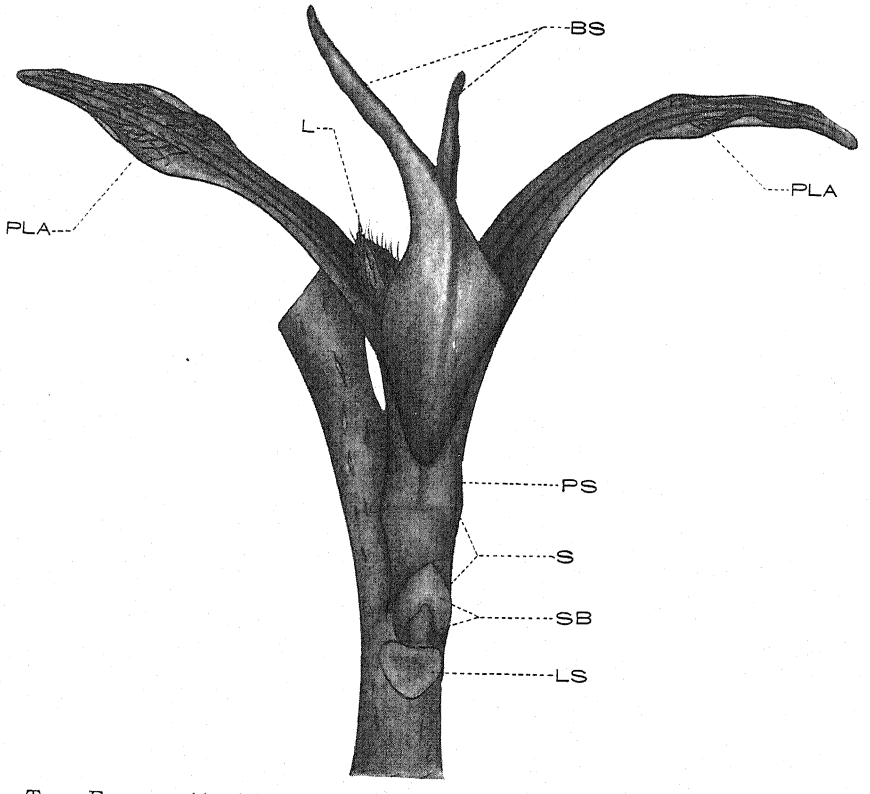
of the prophyll are clearly equivalent to the mid-rib regions of two connate leaves.[15] From this standpoint, the striking contrast between the "monocotyledonous" venation of the prophyll and the palmate-dichotomous venation of the cataphylls is understandable (cf. Foster, 1931, Pl. LXIV.).

PROPHYLLARY BUDS

Cogent evidence of the bifoliar character of the prophyll is furnished by the fact that this organ, in certain cases, subtends two small buds which are laterally placed, one opposite each lateral keel. Prophyllary buds appear to be absent from the buds of spur or slowly-growing shoots but vegetative vigor frequently stimulates their development. Dissection indicated that in most

[15] The arrangement of the veins in each trio is strikingly similar to the venation of the mid-region of the cataphylls of *Ribes* (Bugnon, 1928*a*, p. 43, fig. 2) and *Helleborus* (Glück, 1919, fig. 168 D).

cases these prophyllary buds are early arrested in their growth and that they consist usually of a prophyll and the primordium of the first scale. Every degree of reduction from this stage of development has been found down to the condition where the prophyllary buds are merely indicated by two scarcely differentiated meristematic masses. In the case of precociously expanding axillary buds, however (text fig. 11), the prophyllary buds are much



TEXT FIG. 11. Abaxial view of a stalked axillary bud, expanding precociously on June 5, 1930 (×3). Note that the upper lateral portions of the prophyll have developed into two simple, petiolate leaves, the venation of which is shown in part. For further descriptions see text. *LS,* leaf scar; *SB,* serial buds; *S,* stalk of expanding bud; *PS,* prophyllary sheath; *PLA,* prophyllary lamina; *BS,* scales; *L,* cluster of foliage leaves.

better developed and, in addition to a prophyll, may possess the primordia of four scales. No instances have been observed of shoots developing from prophyllary buds but closer study of injured or defoliated plants may be expected to reveal them.

Proliferation of the Prophyll

In normal buds, the sac-like prophyll shows no evidence of a lamina region and, as I have already pointed out, is non-accrescent during bud expansion in the spring. During the summer of 1930, however, a number of interesting cases of prophyll expansion were noted in precociously expanding buds of certain trees. A typical example is shown in text figure 11. It will be seen that the lateral portions of the prophyll of the short-stalked axillary bud have developed into small, simple petiolate leaves which are joined near the base forming a tubular sheath. The venation of the free, leaf-like portions of this prophyll is highly significant. Each petiolar region is traversed by three strong median veins, a situation obviously comparable with the trio of strong veins found in the keeled regions of the normal prophyll (cf. text fig. 10). The small, unequal, slightly crenate laminae of the proliferated prophyll have a weak reticulate venation, which is derived directly from the median petiolar veins. Thus, in both form and venation, each free leafy portion of this expanded prophyll closely resembles a type of cataphyll occasionally appearing in precociously expanded terminal buds (cf. Foster, 1931, p. 879, and Pl. LXIV, fig. 10).

Although the precise factors causing the proliferation of the prophyll are obscure, certain obvious correlations of growth between the buds of a shoot are worth noting. In all cases observed, the most conspicuous proliferation occurs in the main axillary bud while the prophylls of the smaller serial accessory buds at the same node fail to expand (text fig. 11). This fact is very striking on vigorous coppice shoots where the serial buds are more numerous than on normal twigs. Furthermore, several specimens collected showed a gradual decrease in the degree of proliferation of the prophylls of the successive axillary buds, those near the apex having the normal, sac-like form. In view of such transitional conditions, it seems probable that the difference between the highly expanded prophyll (text fig. 11) and the prophyll of normal buds (text fig. 1) is not qualitative and that hence, as other lines of evidence have shown, the prophyll is truly bifoliar in character.

Ontogeny

Materials and Methods

The material used for developmental studies was collected during 1930 and 1931 from the same localities described in previous papers (Foster, 1931 and 1932) and preserved in a five percent solution of formalin. Owing to the small size of the axillary buds during their early development and the necessity for accurate orientation, a special technique was adopted which represents a modification of the method employed by Luyten and Versluys (1921, p. 90). The young shoot is carefully defoliated under the binoculars, washed thoroughly in water and immersed for about five minutes in a strong

solution of iodine and potassium iodide. These reagents stain the embryonic buds a deep brown and thus facilitate an accurate study of the shape and extent of the foliar initials and the growing point. The stained shoot is then lightly rinsed in water and examined under the microscope. When a decision has been reached as to the desired orientation of a given bud, the shoot is firmly imbedded, in the appropriate position, in a small lump of plasticine and the whole preparation immersed in water in a shallow glass dish for microscopical study.

Prophyll and Scale Ontogeny

The primordia of the new axillary buds begin development during the summer and are clearly seen in the axils of the foliage leaves of the winter bud.[16] At this early stage, the bud primordium is a slightly elevated, bun-shaped mass of meristematic tissue, the longest diameter of which is at right angles to the median plane of symmetry of its subtending leaf (Pl. L, fig. 1). The critical development stages of the prophyll occur during the period of the swelling and expansion of the main bud in the spring. For convenience and emphasis, these ontogenetic stages will be described separately, although in reality the individual stages merge.

Stage 1. The first sign of prophyll development consists in the elevation of the peripheral region of the bud primordium. The lateral and adaxial portions of this peripheral zone develop very rapidly from the start while the abaxial portion appears simply as a slightly elevated rim (fig. 2). As a result of the zonal mode of origin of the prophyll, a central, somewhat depressed mass of embryonic tissue remains undifferentiated and is destined later to function as the growing point, from which the scales and foliage leaves of the axillary bud originate.

Stage 2. During this stage, the apices of the lateral portions of the prophyll grow rapidly and become clearly distinct from the more slowly elevating, notched adaxial region (figs. 3 and 4). As a result of the relatively slow development of the abaxial rim-like portion of the prophyll, the face of the bud becomes more or less sloped in character. This feature is clearly seen when the bud at this stage is viewed from above (fig. 5). From this aspect, it will be noted that the prophyll is deltoid in contour and quite distinct from the central ovate growing point.

Stage 3. Following the stage just described, the apices of the lateral portions of the prophyll begin to converge towards each other (fig. 6). At the same time, active growth ensues on the free abaxial margins. These growth processes are accompanied by the further development of the adaxial portion of the prophyll which is gradually losing its early notched character. The prophyll finally acquires the form of an irregular cup, open in front and partially concealing the growing point. Colleters, which have not been shown

[16] Thus the axillary bud is initiated almost two years before it actually develops into a shoot.

in the illustrations, are developing rapidly at this stage on the lateral and adaxial surfaces of the prophyll while its abaxial face remains glabrous for some time. During or prior to this ontogenetic stage, the embryonic tissue at the abaxial base of the main axillary bud may increase in extent and form a pad-like swelling, which proves to be the primordium of a serial accessory bud (fig. 6). Although the ontogeny of this serial bud has not been followed in detail, its mode of origin agrees with the situation described by Sandt (1925, p. 13, fig. 3) for *Carya glabra* Sweet.

Stage 4. The rapidly converging apices of the lateral portions of the prophyll finally meet (fig. 7). Meanwhile, the adaxial portion has continued to elevate and the free, rapidly-growing abaxial margins are approaching one another. As a result of these growth processes, the prophyll begins to acquire a sac-like form, as is clearly shown in a top view of this stage in development (fig. 8).

Stage 5. During its final critical stages in development, the prophyll increases rapidly in size and its free abaxial margins continue to grow until they meet (Pl. L, fig. 9 and Pl. LI, fig. 10). Continued growth in size, accompanied by the extension of the region below the base of the valvate abaxial margins, leads to the form of the adult prophyll (text fig. 1).

The method of prophyll development just described seems to represent the average or "typical" condition for this species. Deviations from this type of ontogeny occur, however, even in the successive buds of the same shoot. When examined comparatively, these deviations prove to depend upon differences in the *early rates of growth* of the abaxial, adaxial, and lateral portions of the prophyll initial. In certain relatively infrequent cases, the adaxial region of the prophyll grows from the beginning at about the same rate as the apices of the lateral portions. As a result, at a stage comparable to that shown in figure 6 of Plate L, the prophyll has the form of an even-margined cup (open in front) the adaxial portion of which soon acquires a hooded character. A more common deviation is caused by the early rapid growth of the lateral portions of the prophyll and the *delayed development* of its adaxial region. As a result of this pronounced inequality of growth, the adaxial region of the prophyll is deeply cleft at an early stage (Pl. LI, fig. 16). Frequently, as figure 16 illustrates, the abaxial portion of the prophyll is rim-like. In several cases, however, I have found the abaxial bases of the lateral portions distinct from each other, resulting in the appearance of two more or less discrete, adaxially displaced primordia. After the prominent apices and abaxial margins of these primordia have met, they are elevated to varying degrees by the extension of the entire basal zone from which they originated. The degree of this zonal extension results in the variations in the adult form of the prophyll which have already been described.

A still more striking deviation from the usual ontogeny is shown in the method of prophyll development of buds on vigorous coppice shoots. Here the first evidence of foliar differentiation consists in the appearance of a pair

of separate adaxially displaced primordia [17] (Pl. LI, fig. 17). Following a rapid increase in size, the apices of these primordia begin to converge and active growth ensues on *both* their abaxial and adaxial margins, resulting in the formation of wings [18] (fig. 18). Figure 18 shows that the adaxial wings of the primordia are valvate near the base while the abaxial wings are distinctly separated. As development continues, the converging apices meet and the margins of each primordium become slightly involute. Coincident with the development of the bud scales, the adaxial portions of the two prophyllary initials become elevated upon a common zone, while the abaxial margins meet just above the base and may even overlap to some extent. Further increase in size leads to the adaxially-cleft prophyll typical of buds of coppice shoots.

The ontogenetic variations just described seem to indicate definitely that the so-called "lateral portions" of the prophyll initial, in the average condition (Pl. L), correspond to two foliar primordia. However, since the adjacent bases (*i.e.* the "adaxial" and "abaxial" regions) of these primordia are united almost from the start, it would be quite inaccurate to describe their subsequent unit development as an ontogenetic "fusion." The careless use of the terms "fusion" and "concrescence" has been responsible for considerable misunderstanding, particularly in regard to the organogeny of the flower, and the whole controversy has recently been revived.[19] Many writers (*e.g.* Buchet, 1928) in an attempt to avoid the danger of the literal meaning of the term "fusion," have substituted the expression "congenital concrescence." This term, however, is to some extent ambiguous and clearly, at least in the present case, fails to describe adequately the true ontogenetic process.

On the contrary, I regard the peculiar ontogeny of the prophyll as the result of a process of "zonation," comparable to the method of origin of gamosepaly and gamopetaly in many flowers [20] and to the mode of development of the cotyledonary tube in certain spermatophyte embryos.[21] The first evidence of the prophyll is due to the activity of the peripheral tissue of the bud primordium resulting in what I shall term the "prophyllary zone." Invariably two centers of active growth, which are lateral in position, appear early in this prophyllary zone. Dependent upon the rate and intensity of growth at these two points as compared with the intervening regions of the zone, two extreme types of prophyll ontogeny may result, namely, the average condition (Pl. L, figs. 2–5) or the condition of two virtually distinct prophyll initials (Pl. LI, figs. 17 and 18). The significant transitions between these

[17] According to Pax (1890, p. 69) the paired lateral prophylls of dicotyledons are frequently adaxially or abaxially convergent.

[18] Indeed, the form of each of these primordia at this stage closely resembles the initial of an outer scale of the terminal bud (cf. Foster, 1932, pl. II, fig. 5).

[19] Cf. Bugnon (1928*b* and 1928*c*) and Buchet (1928).

[20] Cf. Coulter and Chamberlain (1903, pp. 12–13).

[21] Cf. Coulter and Land (1914, pp. 514–515).

extremes have already been described (fig. 16). The "causal factors" responsible for the variations in prophyll development require closer study. It should be noted, however, that vigorous growth conditions (*i.e.* those obtaining in coppice shoots) usually favor the development of two more or less separate primordia while slower growth seems to result in the more uniform extension of the whole prophyllary zone.

The development of the scales of the axillary bud offers several features of interest. When the prophyll has become closed (Pl. LI, fig. 10), the primordium of the first scale appears as a swelling on the abaxial side of the growing point (fig. 11). This scale initial rapidly increases in size and active marginal and apical growth ensue (fig. 12). During the early process of wing-development in the first scale, the semi-circular pad-like primordium of the second bud scale appears at the adaxial side of the growing point (fig. 13). The basal extent of this primoridum is soon so great that the growing point of the bud is greatly decreased in size. After active marginal growth has commenced in the second scale, the "regenerating" growing point forms a third primordium which is lateral but somewhat abaxially displaced (fig. 14). In the bud illustrated, this initial develops into a cataphyll although, as is shown in text figure 4, in other cases it may form a foliage leaf. Following the appearance of the third scale, the spirally-arranged primordia of the foliage leaves appear (fig. 15). The later critical stages in ontogeny of the foliage leaves and scales are identical with those already described in detail for the terminal bud and require no further discussion here (cf. Foster, 1932).

The assumption has been previously made that the two "median" scales of the axillary bud represent a pair of foliar organs decussate with the two connate phyllomes forming the prophyll. The fact that the primordia of these two scales arise successively rather than simultaneously, as is usually the case with paired leaves (*cf.* Foster, 1929, p. 451 and pp. 459–460), might throw some doubt on this interpretation. However, in a study of scale development in buds of coppice shoots, I have found that the primordia of the inner pairs of scales (*i.e.* the second and third) may arise either simultaneously or successively, the mode of origin varying even in the same bud. Hence the successive origin of the first two bud scales cannot be said to disprove their assumed paired character.

Comparative Studies and Conclusions

The present investigation has led to the conclusion that the prophyll of *Carya Buckleyi* var. *arkansana* is truly bifoliar in organization. This interpretation has been shown to be in strict accordance with evidence secured from the following sources, namely: external form and position, nodal anatomy, venation, prophyllary buds, proliferation of the prophyll, and ontogeny. In view of the fact that a separate evaluation of each of these concordant lines of

evidence has been given, any further discussion of them seems unnecessary at this point.

Since the present investigation has been intensive in character, the results of a brief comparative study of the prophylls of certain other species of *Carya* may be of interest. For the sake of the possible systematic value of these results, the species studied have been grouped under their respective sections.

Apocarya

1. *Carya pecan* Asch. and Gr. The axillary buds of this species are embraced by an ovate-acute, bicarinate, sac-like prophyll.[22] Enclosed within this prophyll and opposite its lateral keels occur at least on vigorous buds, two ternate clusters of staminate catkins.[23] The latter are comparable to the tiny prophyllary buds of *C. Buckleyi* var. *arkansana* and are highly suggestive of a bifoliar type of prophyll. Further evidence for this belief is furnished by the proliferation of the prophyll of buds on coppice shoots. In such cases, two discrete lateral prophylls, each with a lamina of three tiny leaflets, are produced. The ontogeny of the prophyll of *C. pecan* has been found to coincide in all important details with the average type of prophyll development in *C. Buckleyi* var. *arkansana*.

2. *Carya cordiformis* K. Koch. In this species, the axillary buds are provided with two paired valvate prophylls which are only joined at the base.[24] Since it seems probable that the tubular or sac-like type of prophyll in *Carya* may represent a derived condition, the separate lateral prophylls of *C. cordiformis* may typify the primitive condition for this genus. In this connection, it is of interest to note that in *Juglans* and *Platycarya* the axillary buds likewise possess paired lateral prophylls.[25]

Eucarya [26]

Through the courtesy of the curator of the Arnold Arboretum, Mr. Alfred Rehder, a study of several species of this section has been possible. The form of the adult prophyll of these species is fundamentally like that of *C. Buckleyi arkansana*. Certain interesting differences in the ontogeny of the sac-like prophyll, however, are worth recording. In *C. ovata* K. Koch and *C. laciniosa* Schn. the lateral portions of the prophyll initial are extremely prominent and the adaxial region deeply cleft at an early stage, thus resembling one of the

[22] For illustration cf. de Candolle (1862, pl. I, fig. 3), Trelease (1896, Pl. 13, figs. 1–3), and Woodroof (1924, figs. 2, 3 and 4).

[23] The position of these two clusters of staminate catkins on the expanded shoot is illustrated by Engler (1894, fig. 18D) and Woodroof (1924, fig. 6 and 1930).

[24] For illustrations cf. de Candolle (1862, Pl. I, fig. 2) and Trelease (1894, Pl. 13, figs. 10–12).

[25] Cf. de Candolle (1862, Pl. I, figs. 1 and 6).

[26] Cf. Trelease (op. cit. Pls. 14 and 15) for illustrations of buds of species of this section.

common ontogenetic deviations occurring in *C. Buckleyi* var. *arkansana*. The later stages in development agree with the condition in the black hickory. In *C. alba* K. Koch, the ontogeny of the prophyll closely agrees with the situation in *C. Buckleyi* var. *arkansana*. A more specialized type of ontogeny occurs in *C. pallida* Ashe, *C. glabra* Sweet, *C. glabra* var. *megacarpa* Sarg., and *C. ovalis* var. *obovalis* Sarg. In these forms, the lateral and adaxial portions of the prophyllary zone develop at nearly the same rate, resulting in a cup-like primordium which soon acquires a hooded character. This type of prophyll ontogeny seems to be extremely infrequent in *C. Buckleyi* var. *arkansana* and apparently never occurs in *C. alba, C. laciniosa,* or *C. ovata*. Possibly it represents the most highly specialized condition in the genus.

In conclusion, it must be emphasized that further comparative studies are needed before a complete account of prophyll organization in *Carya* can be given. Of the fifteen species of hickory listed by Sargent (1922, pp. 176–177) as native to the United States, only nine have been considered in this paper. An investigation of the prophylls of the other species may be expected to shed more light upon the foliar organization of a genus which still requires further critical taxonomic study.

Summary

1. In *Carya Buckleyi* var. *arkansana* Sarg. the axillary buds on normal spur or long shoots possess a sac-like prophyll which, except for a more or less prominent opening at its abaxial apex, completely embraces the inner organs of the bud. This type of prophyll is unusual in dicotyledons and hence deserves careful morphological investigation.

2. The present study indicates that the prophyll is bifoliar in organization. The evidence given in support of this interpretation may be summarized as follows:

a. The prophyll is often prominently bicarinate, the keels corresponding to the midribs of two connate phyllomes.

b. The prophyll is followed by two median scales which are regarded as a pair of foliar organs decussate with the two laterally connate phyllomes which form the prophyll.

c. The prophyll is supplied by five to eleven separate traces while the bud scales and foliage leaves possess a trilacunar node. Each lateral keel of the prophyll is innervated by a strong unbranched trace which represents a mid-vein.

d. The presence of a trio of strong veins in each lateral region of the prophyll suggests two more or less independent vein systems.

e. The prophyll on vigorous shoots frequently subtends two small buds which are laterally placed, one opposite each keel.

f. Under conditions of abnormal or vigorous growth, the lateral portions of the prophyll may proliferate to form two small simple petiolate leaves which are united at their base forming a short tubular sheath.

g. The prophyll is initiated by the elevation of the peripheral region of the bud primordium. Invariably two centers of active growth which are lateral in position appear early in this "prophyllary zone." The rapid growth of these lateral portions is usually accompanied by the elevation of the adaxial region resulting in the formation of a cup-like primordium which finally becomes closed later in ontogeny. The formation of paired separate prophyll initials on the buds of coppice shoots, together with certain other ontogenetic variations, indicate that the lateral portions of the "normal" prophyll primordium represent two foliar initials. The unit development of the latter is due to a process of "zonation" and is not regarded as a case of "ontogenetic fusion."

3. The two median scales following the prophyll arise successively at the growing point. These organs, as well as the inner scales and the foliage leaves, pursue an ontogeny similar to that of the foliar organs of the terminal bud.

4. The results of a preliminary comparative study have shown a similar type of prophyll organization and ontogeny in *C. pecan* Asch. and Gr., *C. ovata* K. Koch, *C. laciniosa* Schn., *C. alba* K. Koch, *C. pallida* Ashe, *C. glabra* Sweet, *C. glabra* var. *megacarpa* Sarg., and *C. ovalis* var. *obovalis* Sarg. The buds of *C. cordiformis* K. Koch, however, possess paired valvate prophylls, joined only at the base.

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EXPLANATION OF PLATES

All of the drawings were made with the aid of a Spencer binocular dissecting microscope at a magnification of 115.6 diameters. Measurements of buds and foliar primordia were made by means of an ocular micrometer used in connection with a calibrated microscope. For purposes of clarity, trichomes have been omitted from all figures.

PLATE L

Successive stages in the development of the prophyll of buds on normal spur or long shoots. The shaded region at the base of the bud in figs. 1, 2, 3, 6, 7 and 9 represents a portion of the base of the axillant foliage leaf.

FIG. 1. Abaxial view of the primordium of an axillary bud (max. width, 178.75 μ; min. width, 110 μ. × 223).

FIG. 2. Abaxial view of a bud showing the initiation of the prophyll which surrounds a central, slightly depressed mass of meristematic tissue destined to become the growing point of the bud (max. width, 250.25 μ; min. width, 178.75 μ; diameter of growing point, 71 μ. × 160).

FIG. 3. Abaxial view of a later developmental stage, showing the central growing point of the bud surrounded by the rapidly elevating prophyll. Note the prominence of the lateral and adaxial regions of the prophyll (max. width, 321.75 μ; min. width, 250.25 μ. × 115).

FIG. 4. Adaxial view of bud shown in fig. 3, illustrating the notched character of the prophyll at this stage (basal width, 321.75 μ; ht. to base of notch, 143 μ. × 115).

FIG. 5. Top view of bud shown in fig. 3. Note the oval contour of the growing point, the longest diameter (110 μ) being at right angles to the sides of the completely embracing prophyll (× 115).

FIG. 6. Abaxial view of a developmental stage following that shown in fig. 3. The apices of the lateral portions of the prophyll are converging towards each other and active growth is occurring on the free abaxial margins. The pad-like swelling at the base of the prophyll is the primordium of a serial accessory bud (max. width 286 μ; max. ht. 250.25 μ. × 139).

FIG. 7. Abaxial view of a later stage, showing the apices of the lateral portions of the prophyll in contact. The growing point of the bud is partially concealed by the rapidly converging abaxial margins of the prophyll (basal width of prophyll, 357.5 μ; ht. to point of contact of apices, 214.5 μ. × 103).

FIG. 8. Top view of bud shown in fig. 7. Note the greatly decreased prominence of the adaxial notch of the prophyll (cf. with figs. 4 and 5). The growing point of the bud is not visible in this view and the cavity within the prophyll is merely shown in black. (× 103).

FIG. 9. Abaxial view of a developmental stage following that shown in fig. 7. The prophyll has enlarged in size and its rapidly converging abaxial margins have nearly concealed the growing point of the bud (max. width, 357.5 μ max. ht., 286 μ. × 109).

PLATE LI

FIG. 10. Abaxial view of stage following that shown in fig. 9. As a result of the convergence of its abaxial margins, the prophyll has the form of a closed sac (max. width and ht., 500.5 μ. × 77).

FIGS. 11–15. Critical stages in the development of the inner foliar organs of the axillary bud; the prophyll has been removed in every case. The shaded region at the

base of figs. 11–13 represents the position of the lateral region of the prophyll; in figs. 14 and 15, it represents the position of the first bud scale. In all but fig. 11, the significant dimensions of the larger primordia are expressed in terms of basal width × height.

FIG. 11. Lateral view of the growing point (shown at right of figure) and the primordium of the first scale (diameter of g.p., 110 μ; basal width of scale-primordium, 143 μ. × 172).

FIG. 12. Lateral-dorsal aspect of a later developmental stage of the first scale; growing point shown at right of figure (first sc. 143 μ × 178.75 μ. × 139).

FIG. 13. Lateral aspect, as seen from above, of the first scale (shown at the left) and the crescentic, pad-like primordium of the second scale. A portion of the growing point may be seen at the left of the ventral surface of the primordium of the second scale (first sc. 214.5 μ × 143 μ; second sc. 178.75 μ × 71 μ. × 181).

FIG. 14. Ventral aspect of a later developmental stage of the second scale, and lateral aspect of the papillate primordium of the third scale just arising from the growing point (shown in the figure at front and to the right). (Second sc. 150 μ × 143 μ. × 167).

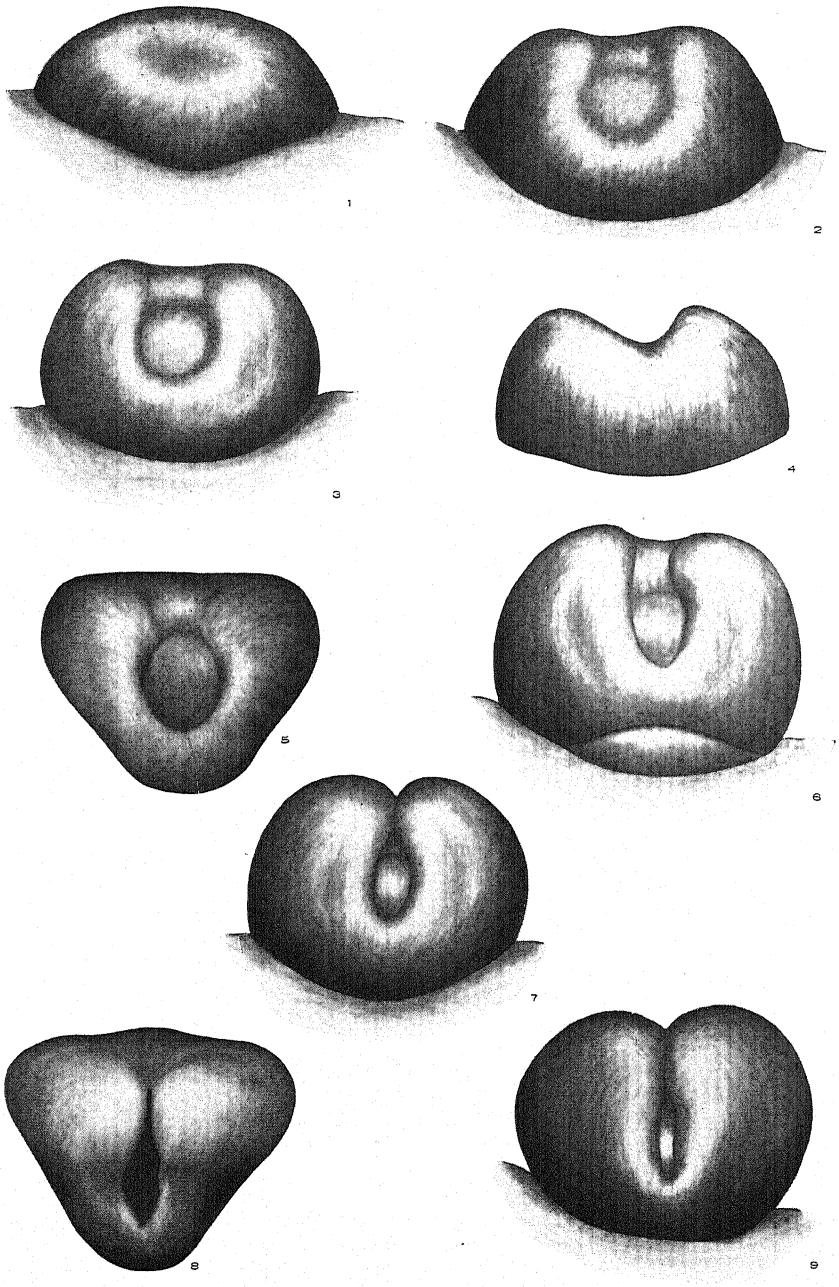
FIG. 15. Ventral aspect of a much later developmental stage of the second scale (which now possesses massive wings) and lateral view of the primordium of the third scale. Partially covered by the left wing of the second scale and overshadowed by the primordium of the third scale, is a tiny foliar initial which probably would have developed into a foliage leaf (second sc. 321.75 μ × 321.75 μ; third sc. 143 μ × 110 μ. × 102).

FIG. 16. Abaxial view of a young axillary bud illustrating a common variation from the type of prophyll ontogeny shown on Plate L. Note that while the abaxial rim connecting the lateral portions is definite, the adaxial region is deeply cleft (max. diameter, 357.5 μ; min. diameter, 250.25 μ. × 98).

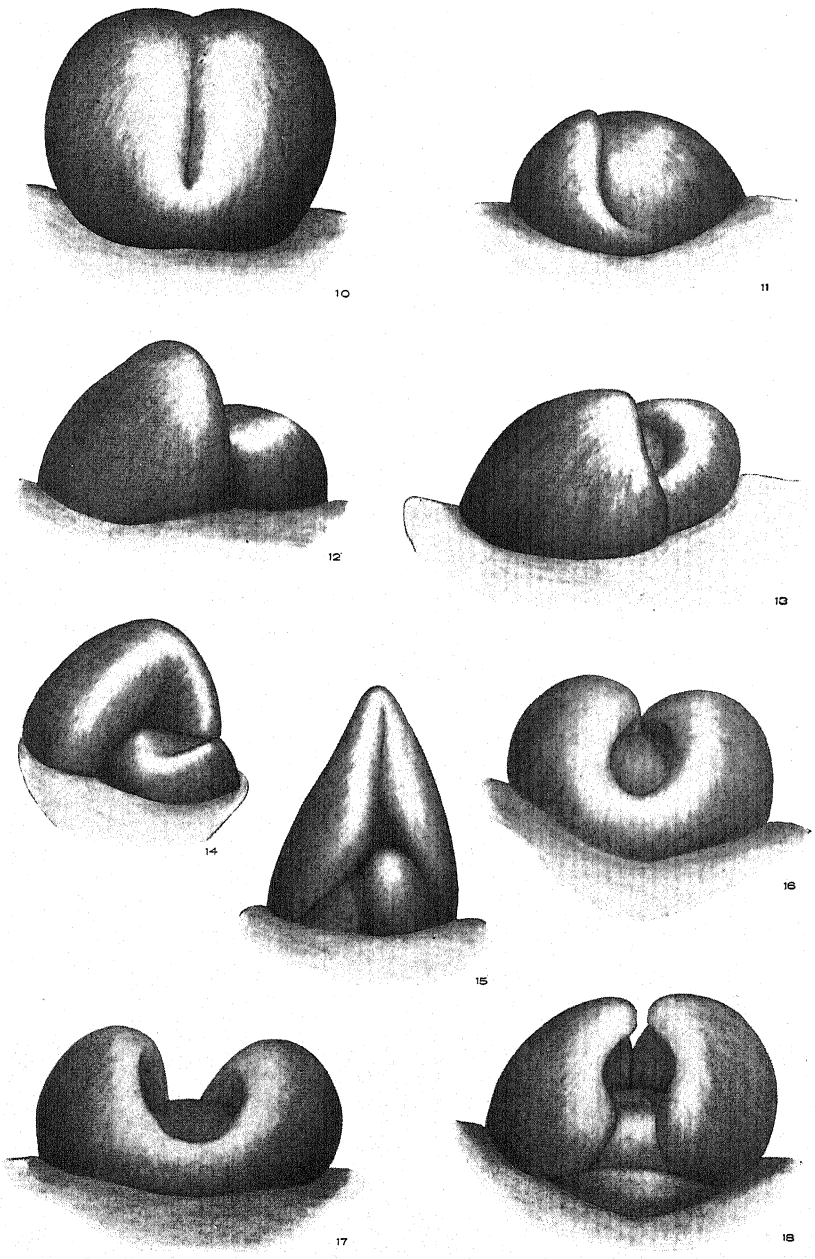
FIGS. 17 and 18. Successive stages in the development of the axillary bud of coppice shoots, illustrating the formation of paired prophylls.

FIG. 17. Very young stage, showing two discrete, laterally-placed prophyll initials, between which is seen the flattened growing point (max. diameter, 321.75 μ; min. diameter, 250.25 μ. × 127).

FIG. 18. Abaxial view of a later developmental stage. Note the adaxial convergence of the two asymmetrical prophylls which are widely separate abaxially and valvate near their bases adaxially. The swelling shown between the abaxial bases of the prophylls represents the primordium of the first scale, posterior to which occurs the growing point of the bud. The cushion-like elevation in front and at the base of the axillary bud is the primordium of the serial accessory bud (max. diameter, 429 μ; min. diameter, 250.25 μ; space between abaxial bases of prophylls, 70 μ. × 88).



FOSTER: PROPHYLL OF CARYA



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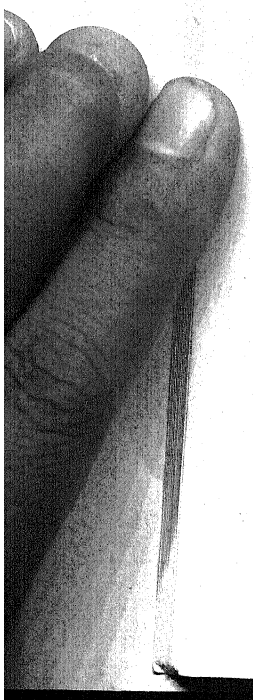

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CONTRIBUTIONS TO THE CHEMISTRY OF THE PLANT CELL WALL VI. FURTHER STUDIES ON THE LOCATION OF LIGNIN IN THE CELL WALLS OF WOOD

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In 1925 Ritter (12) opened a new field of research, by treating thin sections of xylem with some of the reagents commonly used in the chemical analysis of wood. By watching these sections with a microscope during the various treatments, he was able to study the distribution of lignin and cellulose in the cell walls themselves. This was a new departure in the case of wood,[1] since the usual quantitative procedures in which samples of sawdust are used give no indication of the chemistry of the various cell wall layers, or of the different cell types present. The use of certain so-called "microchemical" stains, as indicators of chemical composition in work of this nature, has been reviewed elsewhere (4), and need not be considered in this paper.

With the above technique, Ritter showed that the middle lamella or central layer of the cell wall was lignin, since it was insoluble in 72% sulfuric acid, and soluble when treated several times with chlorine followed by sodium sulfite solution. He concluded, moreover, that the larger portion of the lignin in wood was located in this layer. In the first instance, after the initial reaction of the sulfuric acid, a finely divided, amorphous residue was observed in the place previously occupied by the cell wall layers between the middle lamella and the lumen of the cell.[2] This brownish material was termed "cell wall lignin." Harlow (4) repeated these experiments with thin sections of wood, and corroborated Ritter's observations concerning the middle lamella,[3]

[1] In 1888 Mangin (11) reported the results of numerous experiments on the chemistry of the cell wall, but most of his material was of the non-woody type.

[2] In some instances these may be further differentiated either structurally or in a few instances chemically, into well marked secondary and tertiary layers.

[3] It has been found recently that the middle lamella of the parenchymatous wood ray cells in the sapwood of western yellow pine is soluble in 72 percent sulfuric acid. This interesting exception will be investigated further in connection with the transition between sapwood and heartwood in the hard pines.

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but was unable to find the "cell wall lignin" as described in the original paper. Instead, it was observed that in the hardwoods,[4] the cell wall layers other than the middle lamella swelled rapidly during the acid treatment, and in most cases dissolved completely (Pl. LII, figs. 1–2).[5] The cell walls of the softwoods, in contrast, retained their structural form, and although greatly swollen, did not appear to dissolve as in the hardwoods. Lüdtke (10), and Scarth (14) and his co-workers have recently illustrated this fact in spruce. On the basis of these observations, it was suggested that in the tracheids and fibertracheids of the hardwoods, there was very little if any lignin in the secondary cell walls, while in the softwood tracheids these layers were appreciably lignified. More recently, it has been shown (6) that these differences in behavior may be correlated with some of the physical properties of hardwood and softwood lignin, respectively, as prepared by the usual sulfuric acid method. At this point, it should perhaps be noted that when thin sections of *either* hardwoods or softwoods are treated with the acid and allowed to stand for some time (15 minutes to an hour or more) a finely divided, amorphous, brown residue precipitates out from the previously clear solution (fig. 3). This is presumably the substance referred to by Ritter (12) as "cell wall lignin." However, in the hardwoods, complete dissolution of the cell walls occurs during the first few minutes of the acid reaction, while this amorphous deposit does not appear until sometime later. There is, therefore, no way of knowing from what portion of the cell wall it originated. Whether or not it can be classified as "lignin" will be considered in another section.

On the assumption that cell wall lignin occurred as previously described, Ritter (l. c.) devised a technique for separating this form of lignin quantitatively from that residual in the middle lamella. Small cubical blocks, 3 mm. on edge, were prepared from the wood of several species, and after extraction with alcohol-benzol, were treated for 24–36 hours with 72 percent sulfuric acid. The diluted wood-acid mixture was then boiled for five hours, and the finely divided, dark brown amorphous material ("cell wall lignin") thus obtained was separated mechanically from the blocks of "middle lamella lignin." These were then boiled in water, with intermediate washings, for a total of 16 hours. The additional residues thus separated were added to that obtained at first, and the sum total weighed as "cell wall lignin." Although Harlow's (4) experiments were confined to thin sections, he pointed out that the prolonged boiling of the blocks is "an extremely drastic treatment of the fragile structures remaining after digestion in concentrated acid;" Freudenberg and his co-workers (3) have also criticized this technique for similar reasons. Since then considerable work has been done with small blocks of this sort and the results of these experiments are given below.

Quantitative Experiments with Small Blocks

At the start, it was suspected that the fine amorphous residue which appeared upon dilution of the wood-acid mixture, might not be the same as

[4] Hardwoods—dicotyledonous trees; softwoods—coniferous trees.

[5] See original paper for differences in the behavior of individual species.

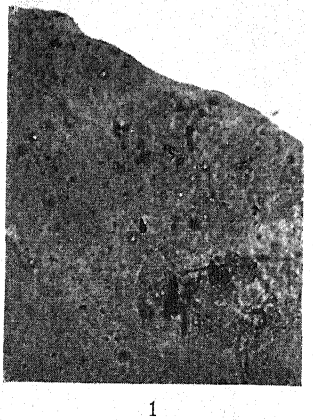
that derived from the subsequent boiling of the blocks. These two "fractions" were, therefore, kept separate from each other. In the initial treatment, the blocks were allowed to stand for 48 hours in the concentrated acid at room temperature. The resulting dark colored mixture was then diluted and brought to a boil. After partial settling had taken place, the blocks were carefully removed and washed by decantation. The copious brown, flocculent residue, "cell wall lignin," remaining in suspension was then collected on matched filters (7) and, incidentally, proved exceedingly difficult to wash, on account of its somewhat gelatinous nature. The blocks were then boiled under a reflux in the diluted acid filtrate for five hours, and finally for sixteen

TABLE 1. *Percentage of Lignin in Two Woods, Sampled as Small Blocks*

Species	Lignin in Small Blocks	1st Residue, After Dilution (Amorphous)	Lignin of 2d Residue, After Boiling (Structural)	Total Amount of "Lignin"
Red Alder (*Alnus rubra* Bong.)	22.7	2.3	.3	25.3
	22.5	2.8	.3	25.6
Western Yellow Pine (*Pinus ponderosa* Laws.)	26.0	2.2	.1	28.3
	26.1	3.3	.1	29.5

hours in water, as specified by Ritter. The fine residue thus collected was tared separately on matched filters. The lignin yields of the blocks and of the first and second residues, respectively, from red alder and western yellow pine, are recorded in table 1.

Of much more importance than these quantitative yields is the fact that the undried residues proved to be quite different from each other when viewed



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TEXT FIG. 1. Amorphous material obtained quantitatively from the diluted wood-acid mixture. (Background opaqued for better contrast.) × 430.

TEXT FIG. 2. Amorphous material obtained by treating cotton cellulose with 72 per cent sulfuric acid at room temperature for 36 hours. Compare with text figure 1. × 225.

with the microscope. The flocculent substance which precipitated at first was amorphous in character, and was apparently identical with that appearing when thin sections were allowed to stand in small amounts of concentrated acid for a considerable time (text fig. 1). In contrast, the residues from the boiled blocks were definitely *structural* in nature, and simply consisted of small pieces which had been removed mechanically by this prolonged treatment (fig. 4).

It would appear, therefore, that Ritter's "cell wall lignin" consists of at least two different types of material which he presumed to be identical. For this reason, the relative quantitative yields of "cell wall lignin" and "middle lamella lignin" which he reported in a subsequent paper (13) have questionable significance, since smaller blocks were used and the total surface area was thus increased. This would allow more particles to be separated by the boiling treatment, and would increase the quantity of the "cell wall lignin" obtained. The methoxyl content ascribed to such "cell wall lignin" may perhaps be explained by the fact that in Ritter's technique, portions of the blocks are removed by boiling and are intermixed with the amorphous material obtained at first.

Table 2 shows the amount of "block lignin" and amorphous material obtained by the writer from several woods. In every case the wood-acid mix-

TABLE 2. *Percentage of "Block Lignin" and Amorphous Material from Several Woods*

Species	Lignin in Small Blocks	Amorphous Material	Total Amount of "Lignin"	Lignin in Sawdust from Same Sample
Red Alder (Different sample from that in Table 1)	26.6	3.9	30.5	29.7
	26.5	3.9	30.4	29.8
Basswood (*Tilia glabra* Vent.)	24.6	2.2	26.8	24.3
	24.7	2.2	26.9	24.2
Red Gum (*Liquidambar styraciflua* L.)	27.4	4.5	31.9	30.5
	27.5	4.6	32.1	——
Douglas Fir (*Pseudotsuga taxifolia* Britt.)	35.6	.4	36.0	35.3
	35.5	.4	35.9	——
White Fir (*Abies concolor* Lind. & Gord.)	33.4	.5	33.9	——
	33.1	.5	33.6	——

ture was diluted, brought to a boil, and allowed to settle. The blocks were then removed and washed, as indicated above, and no further hydrolysis was undertaken. Parallel determinations with sawdust from the same samples are also included for comparison.

THE MICRO-STRUCTURE OF THE ACID TREATED BLOCKS

To discover the effect of the concentrated acid on the interior of the small blocks, Ritter (12) allowed the washed samples to dry out to about fiber saturation point (30 percent moisture content), and then impregnated them with melted paraffin. Thin microtome sections were prepared from the cooled,

solidified matrix with its imbedded material. This technique was followed in the present instance, and Plate LIII, figure 5, depicts a section of a red alder lignin block prepared as indicated in Ritter's original paper. Unfortunately, when the blocks are dropped into the melted paraffin, a sudden and marked shrinkage takes place, and sections cut from such material do not show the structural details originally present before their immersion in the embedding medium. In contrast, the following schedule permits of slow infiltration of the blocks at room temperature, and preserves more nearly the natural appearance of the tissues.

EMBEDDING SCHEDULE FOR SMALL BLOCKS OF LIGNIN [6]

1. Wash the blocks thoroughly by decantation.
2. Place them in a cartridge vial; cover with absolute ethyl alcohol, and aspirate for 15 to 20 minutes.
3. Repeat using a fresh change of alcohol.
4. Change the alcohol, stopper the vial, and allow it to stand for 20–30 minutes.
5. Pour off the alcohol and substitute a 50–50 percent mixture of alcohol and xylol (milkiness at this point indicates incomplete dehydration and is ruinous; however, too long immersion in alcohol will result in a partial dissolving of the lignin and must be avoided).
6. Substitute pure xylol, and after one to two hours add some small shavings of paraffin. As these disappear, add more until the additional paraffin falls to dissolve at room temperature. Then place the vial in an oven which is regulated at the melting point of the paraffin. Add paraffin in excess and allow it to dissolve or melt at the temperature of the oven.
7. Pour off the xylol-paraffin mixture and substitute pure melted paraffin. Decant this immediately, taking care to remove the "scum" which sometimes appears at the surface and which contains a large share of the xylol present. Substitute fresh paraffin.
8. After one or two more changes (2–3 hours apart) the material should be entirely free from xylol (this is necessary for successful sectioning later).
9. Pour out the melted paraffin and blocks into a paper tray, and after arranging the material, cool quickly on a cold plate, or float the tray on cold water.

Figure 6 shows a transverse section of a block of red alder lignin from the same sample as that depicted in figure 5 but embedded gradually, using the technique just given. It is evident that not only the middle lamella, but also the other layers of the cell wall still retain their structural identity. That this should be true in the softwoods (fig. 7) was not surprising, since thin sections behaved in a similar fashion, but with the hardwoods it was an altogether unlooked for result. Objections may be raised that the acid did not penetrate completely, and that the cell walls as seen with the microscope still contained part of the undissolved polysaccharides. This does not seem probable for the following reasons:

1. Thin sections of the acid-treated blocks (after removal of the paraffin) were completely soluble when treated several times with bromine followed by weak ammonia (one of the quantitative methods for the removal of lignin from wood).

[6] Small blocks of wood from which the polysaccharides have been removed by acid treatment, as previously described.

2. The sections did not transmit polarized light.

3. Sections cut from the outer surfaces of the blocks where initial contact was made with the acid, appeared very similar (except for greater swelling) to sections cut from the interior.

4. The quantitative yields of lignin from sawdust samples of the same piece of wood were only slightly lower than those of the blocks.[7]

In both hardwoods and softwoods, however, the relative densities of the middle lamella and the remainder of the cell wall appear to be quite different. This was demonstrated by dissolving with xylol the paraffin from the lignin sections, and replacing this solvent with a few drops of absolute alcohol. A section was then observed with the microscope while the alcohol evaporated from the slide, and as the mount dried, a remarkable change took place. The thick, much swollen walls began to shrink, and within a few seconds had almost disappeared except for the network of the middle lamella. Figure 8 illustrates this condition and shows the shrunken remains of the secondary cell walls. Whether the remaining network represents more than the original "middle lamella" cannot be so easily determined, but in any event, the secondary layers seem to contain only a very small portion of the total lignin of the wood.

Discussion

It should be clear from the above experiments that in both the hardwoods and softwoods studied, the secondary walls of the principal longitudinal elements contain small but appreciable amounts of lignin. Moreover, when the proper technique is employed, this portion of the lignin in wood remains *in situ* in the cell wall, and apparently does not lose its structural configuration. Ritter's (12) interpretation, as previously indicated, is based upon the appearance of material embedded in such a way as to alter, drastically, the structural features still remaining after acid treatment.

The occurrence of the dark brown amorphous material, however, has yet to be explained. Of the greatest importance to this discussion is the recent experimental work of Sherrard and Harris (15), in which they show the effect of time, concentration, and especially temperature upon the quantitative yields of lignin as obtained by the usual sulfuric acid method. In this paper they state: "The comparative ease with which carbohydrates may be converted into a lignin-like substance at elevated temperatures suggested that the same effect may be produced when sulfuric acid comes in contact with the wood." Preliminary experiments were performed with solutions of cellulose in 72 percent sulfuric acid, and they found that after 36 hours standing at 25° C., 3 percent of a dark colored lignin-like substance remained after dilution and

[7] The higher lignin yields of the blocks may be due to incomplete hydrolysis of the dissolved polysaccharides near the center. This could probably be avoided by boiling the dilute acid mixture for several hours as suggested by Ritter (loc. cit.). This would, of course, as already noted, also remove small particles from the blocks. However, sections from blocks prepared according to Ritter's technique appeared in every way comparable with those from blocks which had not received the prolonged boiling treatment.

hydrolysis. This substance after chlorination was soluble in sodium sulfite solution. Previous to the appearance of this paper, similar qualitative experiments with cellulose were performed in this laboratory, and text figure 2 shows, at high magnification, some of the charred material obtained. By visual comparison it seems to be very similar to the "dark brown amorphous material" from wood, mentioned earlier. From the foregoing experiments it seems quite probable that Ritter's "cell wall lignin" is a mixture of this carbonized material from the polysaccharides of the cell wall plus portions of the lignin removed from the blocks by vigorous boiling.

Sherrard and Harris (15) state that 70 percent sulfuric acid is preferable to 72 percent; that the temperature should be maintained at approximately 10° C.; and that the time should be limited to 7–16 hours. Under these conditions, the lowest yields of lignin, and the highest yields of reducing sugars were obtained, simultaneously, and the isolated lignin had a high methoxyl content. They conclude that "if the percentage of methoxyl in the isolated lignin may be considered as a check on the purity, sulfuric acid under the controlled conditions produces lignin of higher quality than that produced by other methods."[8]

The quantitative yields of amorphous material (charred polysaccharides) recorded in table 2 suggest that the previous percentages of lignin which other workers have obtained by the usual method at room temperature are all too high. This is true since in several species of wood, the charred material may amount to 3–4 percent of the oven-dry weight of the sample. Careful adherence to the proposed new technique is therefore necessary if accurate lignin yields are desired.

The shrinkage experiments with thin sections of lignin seem to strengthen still further Ritter's (12) original conclusion that most of the lignin in wood is located in the middle lamella. The lignin of the secondary walls, although apparently appreciable in amount, upon drying, actually represents but a small fraction of the total lignin in the section. This fact may perhaps answer Scarth's (14) objection to Ritter's contention. Unfortunately at the present time no technique is known to the writer for separating, quantitatively, the lignin of the middle lamella from that of the remainder of the cell wall. Until this is accomplished, the exact proportion of lignin in the various layers of the cell wall must remain largely a subject for conjecture. It may be of in-

[8] Small blocks, sawdust, and thin transverse sections of several hardwoods and softwoods were treated according to this new technique and the residues examined with a microscope for evidences of carbonized material. In the softwoods, the concentrated acid darkened almost imperceptibly if at all during the 16 hours treatment, and only the smallest traces of amorphous material were subsequently found. In the hardwoods, small amounts of amorphous material were obtained when sawdust or thin sections were used, but this may represent the reprecipitated lignin of the secondary wall. Small blocks of basswood and red gum were not thoroughly penetrated by the acid after 16 hours immersion. Longer immersion resulted in an appreciable darkening of the solution. Carbonization seems to take place more readily in the hardwoods than in the softwoods, and should be investigated further.

terest to note that the lignin of the secondary wall is less resistant to delignifying reagents than that of the middle lamella. This was pointed out for the softwoods in a previous paper (4) and was confirmed with the hardwoods used in the present investigation. Lüdtke (10) is of the opinion that all of the lignin in the woody cell wall is located in the middle lamella, and believes that the acid-resistant secondary wall is composed of a chemically different foreign substance. While it is true that this layer (or series of layers) is more easily attacked by certain lignin solvents than is the middle lamella, it does not necessarily follow that these two adjacent portions of the cell wall are different chemically. As already indicated, the secondary layers seem to be exceedingly porous or sponge-like in character, and presumably permit a more rapid penetration of the various lignin solvents than does the more compact middle lamella.

The results of the above experiments with small blocks can not be reconciled with a previous statement (4) that the cell walls of the hardwoods contain practically no lignin. Objections raised at the same time concerning the efficacy of the Maüle test in microchemical analysis also lose part of their validity.

The structure of the cell wall lignin presents a number of interesting problems some of which will be the subject of further research. It would seem especially desirable to correlate, if possible, the swollen lignin from which the cellulose has been dissolved, with the fusiform units and fibrils of the cell wall as described by Ritter (12), and figured in a three-plane sketch by Scarth (14). It is probable that a study of the porous structures of the wall indicated in figures 7 and 8 may hold the answer to this problem. From the behavior of the secondary cell walls, it may be assumed that the lignin remaining in them after the acid reaction, consists of particles which are very loosely held together. It also seems evident that in the hardwoods studied, the structure of the cell wall is more open than in the softwoods. This is shown by the striking differences in behavior of thin sections or sawdust from the two types, respectively, when treated with concentrated sulfuric acid. The differences are less marked when small blocks are used because the penetration of the acid is slower and the mass of the block reinforces the individual cells, thus preventing them from bursting during the removal of the polysaccharides. The differences in relative porosity are evidenced in figures 7 and 8, but similar sections must be observed and photographed using the highest magnifications available, before we may draw any satisfactory conclusions.

It will also be necessary for investigators to experiment with a relatively large number of woods before any general statements can be made concerning the chemistry and structure of the cell walls of xylem. This has been illustrated in part by the recent work of Dadswell (2) in which he shows a section of an Australian hardwood treated with 72 percent sulfuric acid. In this species a lignin pattern remains which is not entirely comparable with that of our native woods observed thus far. At present it is necessary to use

woods which can be sectioned without preliminary softening in hydrofluoric acid, since this reagent changes certain chemical properties of the sample (4). This situation is a handicap because it is almost impossible to section many native and foreign woods without first softening them. If such a method can be found which does not at the same time alter the wood chemically, the material available for research of this nature will be greatly increased. In future work it would also seem advisable to observe the effect of the various reagents not only upon thin sections but also upon small blocks as suggested by Ritter (12).

The concept of "ligno-cellulose" as originally proposed by Cross and Bevan (1) and adhered to by a number of investigators should perhaps be discussed in connection with the results of the present experiments. According to this theory, the cellulose and lignin in the cell wall are combined chemically and this complex substance is termed "ligno-cellulose." König and Rump (9) objected to this explanation as applied to wood, since they found that sections treated with various reagents still retained their structural features. This position was further strengthened by Ritter's (12) work on the middle lamella, although the concept of a chemical union between the lignin and cellulose of the secondary cell wall was not necessarily imcompatible with his results. The experiments recorded in this paper seem to disqualify still further the term "ligno-cellulose" as it refers to wood. Instead, a structure must be visualized from which either of the two principal components may be removed without destroying the general configuration of the one remaining. Freudenberg (3) has recently described in considerable detail the structure and chemistry of the cell wall, and has arrived at a similar conclusion, while Lüdtke's (10) experiments also serve to strengthen this position.

Scarth (14) states "that the remarkable resistance of medullary ray cells may be due to a high lignin content. . . ." This fact had already been pointed out in an earlier paper (5) in which the wood rays of two species of hardwoods were dissected and analyzed quantitatively. Scarth also states that the color reaction of wood with phloroglucin is due to small amounts of aldehydic substances in the wood. Hoffmeister (8) has shown that this color test is due to the presence of small quantities of a derivative of coniferyl aldehyde.

Summary

1. The contention that most of the lignin in wood is located in the middle lamella is further substantiated.

2. With the proper technique, the lignin of the secondary walls of most of the longitudinal elements remains *in situ* after treatment with concentrated sulfuric acid.

3. This cell wall lignin is exceedingly porous and is loosely held together; it is apparently less dense in the hardwoods than in the softwoods (this applies only to the species studied, although it may be true of many others as well).

4. "Cell wall lignin" as previously described by Ritter consists of the charred decomposition products of the polysaccharides of the cell wall in addition to fragments removed from the lignin blocks by prolonged boiling.

5. Lignin prepared with 72 percent sulfuric acid at room temperature may contain as much as 3–4 percent of charred material from the polysaccharides of the wood. This may probably be obviated or reduced by the use of Sherrard and Harris's recently proposed modification of the method for isolating lignin.

6. Future research of this nature should take into consideration the physical changes which accompany the removal of the cellulose from the cell wall when such reagents as concentrated sulfuric acid are used.

7. Further evidence has been presented to show the defects in the "lignocellulose" concept as applied to wood.

Thanks are due to Drs. H. P. Brown and L. E. Wise, and to Mr. Floyd C. Peterson for helpful suggestions.

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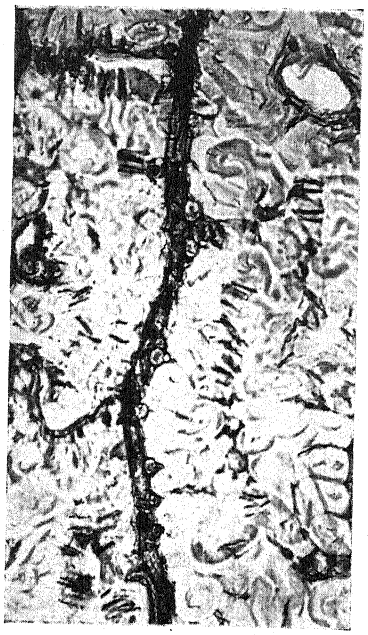

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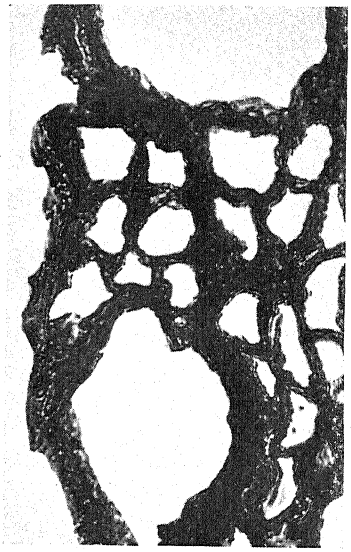

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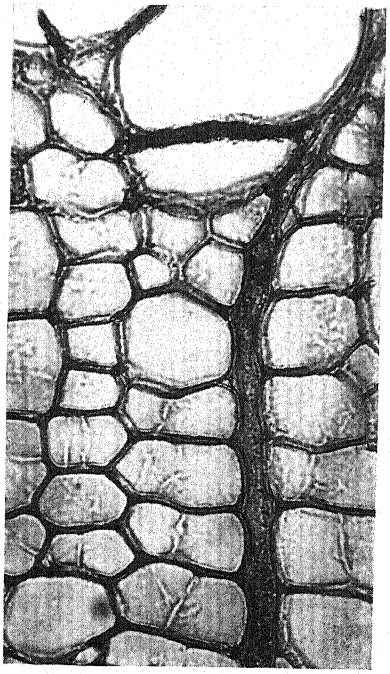

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DESCRIPTION OF PLATES

PLATE LII

FIG. 1. A transverse section of red alder wood during treatment with 72 percent sulfuric acid. × 175.

FIG. 2. The same after complete dissolution of the cellulose. × 175.

FIG. 3. The same after standing for one hour. Note the reprecipitated amorphous material. × 175.

FIG. 4. Structural remnants obtained by boiling the small blocks of lignin for several hours. × 430.

PLATE LIII

FIG. 5. A transverse section cut from a block of red alder lignin embedded in melted paraffin. Note the shrunken condition of the cell walls. (Background opaqued for better contrast.) × 500.

FIG. 6. A transverse section from a block of the same sample, embedded gradually as described in the text. × 500.

FIG. 7. A transverse section from a block of western yellow pine lignin, embedded in the same manner. ×500.

FIG. 8. A similar section to that in figure 6; after the removal of the paraffin, the section was allowed to dry from absolute alcohol. Note the shrivelled remains of the secondary walls. × 500.

SOME UNDESCRIBED FLOWERING PLANTS FROM SOUTH AMERICA

H. A. GLEASON

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Dichorisandra macrophylla sp. nov. Caule crasso prostrato ad nodos radicante, internodiis elongatis, superne adscendente; foliis confertis anguste ellipticis acuminatis basi cuneatis in petiolum latum utrinque minute pubescentibus; panicula unica terminale multiflora, pedunculo valido; bracteis linearibus pedicellos aequantibus; sepalis lateralibus oblique ovatis, posteriore rotundato; petalis posterioribus late obovatis connatis, anteriore obovato saepe 3-lobato; staminibus quadrimorphis; ovulis 4–5 in quoque loculo.

Stem (or rhizome) stout and fleshy, prostrate, rooting at the nodes, with elongate internodes, assurgent and leafy at the summit; leaves crowded, narrowly elliptic, 20–30 cm. long, 7–10 cm. wide, acuminate, cuneate at base into a broad sheathing petiole, very minutely pubescent on both sides; panicle solitary, terminal, many-flowered, 5–8 cm. long, on a stout, minutely pubescent peduncle 5 cm. long; bracts linear, 3–5 mm. long, equaling the pedicels; flowers zygomorphic; lateral (anterior) sepals obliquely ovate, obtuse, freely veined and black-dotted, 9.5 mm. long; posterior sepal 11.5 mm. long, folded into 2 semicircular halves; posterior petals coherent for two thirds of their length, very broadly obovate or subrotund, slightly oblique, with the more concave side anterior, 12 mm. long, freely veined and black-punctate; anterior petal obovate, 10 mm. long, often 3-lobed; posterior stamen: filament very short, often narrowly winged; anther linear-oblong, somewhat falcate and incurved, 4.5 long; postero-lateral stamens: filament very broad and flat, with a conspicuous midnerve; anther narrowly triangular, 5 mm. long, the pollen-sacs divergent and unequal at base; antero-lateral stamens: filament flat, 4 mm. long; anther oblong, 2 mm. long, obtuse or retuse, the two thecae equal in size but the posterior always higher than the anterior; anterior stamen: filament intermediate in length, reverse-triangular, the wing broadening above and continued along one side of the anther nearly to its summit; anther narrowly ovoid, 5 mm. long, slightly falcate and incurved; all anthers opening by 2 minute terminal pores; ovary subglobose, 3-celled, with 4 or 5 ovules in each cell; style cylindric, 6.5 mm. long.

Type, *Ynes Mexia 4403*, collected on the lands of the College of Agriculture at Viçosa, Minas Geraes, Brazil, alt. 670 m., and deposited in the herbarium of The New York Botanical Garden. The collector describes it as a perennial herb growing in dense shade in rich humus, the flowers bright blue, the leaf purple beneath and striped with lighter green above. She also collected it as number *4339*, in bud some two weeks earlier, and as no. *4648a*, in fruit two months later.

Tibouchina triflora sp. nov. § *Diotanthera:* caulibus herbaceis erectis papilloso-villosis, internodiis elongatis; petiolis brevibus strigosis; foliis lan-

ceolatis acuminatis integris basi subrotundis 7-nerviis utrinque dense strigosis; cymis terminalibus et ex axillis superioribus, 3-floris longipedunculatis, pedicellis lateralibus terminalem excedentibus; floribus 5–6-meris; sepalis erectis persistentibus lineari-subulatis hypanthium anguste campanulatum subduplo excedentibus; staminibus forma similibus magnitudine dissimilibus, connectivo brevissime producto crasso U-forme, filamentis glabris; ovario 5–6-loculare supra hispido ad apicem 5–6-lobato; stylo glabro, stigmate punctiforme.

Herbaceous, erect, sparsely branched; stems nearly terete, densely villous with papillose hairs, the internodes as much as 11 cm. long; petioles 2–6 mm. long, densely strigose; leaf-blades thin, lanceolate, 5–9 cm. long, 1.5–2.5 cm. wide, long-acuminate, entire, broadly obtuse to subrotund at base, 7-nerved, closely strigose on both sides, especially on the veins beneath, inflorescence of three 3-flowered cymules, terminal and from the uppermost axils; peduncles 3–5 cm. long, pubescent like the stem; pedicels of the terminal flowers 3–5 mm., of the lateral 10–20 mm. long; flowers 5–6 merous; hypanthium narrowly campanulate, 5.5 mm. long to the torus, about 3 mm. in diameter, densely hispid with light-brown erect hairs 1.2–1.5 mm. long; calyx-tube nearly a millimeter long, sepals erect, herbaceous, persistent, linear-subulate, 8.3 mm. long, 2.3 mm. wide at base, thence abruptly narrowed to 1 mm., hirsute like the hypanthium; petals magenta, obliquely obovate, 9 mm. long, 8 mm. wide; stamens similar in form but of two sizes; filaments 3.8–4 or 4.3–4.5 m. long, glabrous; anthers linear-subulate, 3–3.3 or 3.7–4.2 mm. long, opening by a terminal pore; connective very stout and U-shaped, the basal lobes curved-erect, 0.7 mm. long; ovary 5–6-locular, mostly free, glabrous below, densely hispid above, prolonged at the summit into 5–6 erect finger-shaped lobes surrounding the base of the style; style slender, glabrous, 7.5 mm. long; stigma punctiform, not expanded.

Type, *Killip 7925,* collected at "La Gallera," Micay Valley, Dept. El Cauca, Colombia, alt. 1800–1900 m., and deposited in the herbarium of The New York Botanical Garden; *Killip 7690* was collected in the same place but at 400 m. lower elevation. About a third of the flowers are 6-merous. In foliage the species is completely distinct from all other species of the section. According to the arrangement of the genus by Cogniaux, it would be placed near species 120–122, *T. Rusbyi, T. mollis,* and *T. Solmsii.*

Meriania axinaeoides sp. nov. § *Umbellatæ:* arbor ramis glabris obscure angulatis; petiolis glabris brevibus; laminis firmulis anguste obovatis abrupte et breviter acuminatis integris ad basin longe cuneatis 5-pli-nerviis supra glabris subtus reticulatis ad nervos 3 majores breviter pilosis; panicula pedunculata submultiflora; floribus pedicellatis 5-meris; hypanthio late hemisphaerico glabro; calyce ad anthesin irregulariter fisso lobis ovatis vel rotundatis; dentibus exterioribus parvis conicis; petalis rubris obovatis; staminibus aequalibus; antheris subulatis arcuatis, connectivo ad basin incrassato in calcarem brevem producto; ovario libero ovoideo corona ornato.

Petioles 1 cm. long; leaf-blades 10–18 cm. long, 4.5–6 cm. wide, the veins impressed above, elevated beneath, the tertiaries prominently reticulate; inflorescence glabrous, 8 cm. long, about 20-flowered, its branches strongly angular; pedicels 5–10 mm. long; hypanthium 4.3 mm. long to the torus; calyx widely spreading at anthesis, its lobes 3 mm. long, subcoriaceous; external teeth 1–1.5 mm. long, at the center of the lobes; petals 9–10 mm. long; filaments flat, 8 mm. long; anthers 8–9 mm. long, the basal spur triquetrous,

acute, 2 mm. long; ovary 4 mm. long, 10-ribbed, surmounted by a corona of linear-subulate scales 1–1.5 mm. long; style slender, 8.5 mm. long; stigma punctiform.

Type, *Buchtien 1001,* collected at San Carlos, in the Mapiri region, Bolivia, alt. 850 m., and deposited in the herbarium of The New York Botanical Garden. Its short exterior teeth distinguish it from all described species of the section except *M. quintuplinervis* Naud., a Colombian species in which the leaves are rounded or subcordate at base and the hypanthium densely pubescent.

Graffenrieda stellipilis sp. nov. Arbor parva; caulibus petiolis inflorescentia et hypanthiis arcte stellato-tomentellis; laminis ovato-oblongis chartaceis acuminatis integris basi subrotundatis 3-nerviis supra juventute stellatis mox glabrescentibus subtus ad paginam sparse ad venas dense stellatis; floribus sessilibus 5-meris; calyce subtruncato minute 5-dentatis; petalis extus sericeis; antheris basi calcaratis; ovario 3-loculare villoso; stigmate punctiforme.

Tree 4 m. high; young branches strongly compressed, densely and closely tomentulose with brown stellate hairs; petioles stout, 2–3 cm. long, pubescent like the stem; blades thin, ovate-oblong, as much as 16 cm. long by half as wide, acuminate, entire, rounded or broadly obtuse at base, 3-nerved, the upper side thinly stellate when young, soon becoming glabrous on the surface, persistently stellate on the midvein, the lower side sparsely stellate on the surface, densely so on the midvein; veins plane above, elevated beneath, the secondaries 5–10 mm. apart; panicle small, freely branched, pubescent like the stem; flowers sessile, 5-merous; hypanthium campanulate, about 2 mm. long to the torus, densly stellate; calyx-tube prolonged 1.3 mm., essentially truncate, the sepals represented by 5 minute teeth; petals (immature) sericeous externally; anthers with a conspicuous dorsal spur; ovary free, 3-celled, villous above, 10-alveolate; style straight; stigma punctiform.

Type, *Buchtien 7407,* collected at the Hacienda Casana, above the road to Tipuani, Bolivia, alt. 1400 m., and deposited in the United States National Herbarium, no. 1399156. *Buchtien 7408,* collected in the same locality six weeks later, exhibits the fruit enclosed by an ovoid-urceolate hypanthium; its leaves are somewhat larger, reaching a length of 21 cm.

Most species of *Graffenrieda* have well differentiated sepals, usually triangular in shape, but even the youngest buds on the type of *G. stellipilis* show only minute teeth. It does not appear to be related to the other 5-merous species nor to the small group with pubescent ovary, but resembles more closely *G. parviflora* Cogn., a Bolivian species with sparsely pubescent stem, pilose midvein, and nearly glabrous hypanthium.

Miconia pubicalyx sp. nov. § *Jucunda:* ramis gracilibus 4-angulatis tenuiter arcteque cinereo-tomentellis; petiolis brevibus tomentellis; laminis subcoriaceis lanceolatis acuminatis integris basi rotundatis 3-pli-nerviis, supra glabris atroviridibus subnitentibus, subtus tenuiter arcteque cinereo-tomentellis; panicula mediocre axibus 4-angulatis tomentellis, bracteis minutis late ovatis; floribus 5-meris sessilibus subsecundis; hypanthio campanulato tenuiter stellato-pubescente; calyce subpatente sinubus acutis intus inter lobos pu-

bescente; sepalis triangularibus acutis, dentibus exterioribus minutis patulis subulatis; petalis pro rata magnis elliptico-oblongis oblique retusis ad marginem glanduloso-ciliatis; staminibus dimorphis; filamentis gracilibus glabris; antheris subulatis arcuatis poro minuto dehiscentibus 2-locularibus; connectivo basi dilatato in ser. ext. in lobos 3 subaequales, in ser. int. in lobos 2 laterales rotundatos et lobum 1 dorsalem minutum; ovario 3-loculare ellipsoideo semi-infero; stylo elongato gracile; stigmate minuto truncato.

Stems woody, sharply 4-angled above with flat sides, closely and finely cinereous-tomentulose, the internodes mostly 3–5 cm. long; petioles rather stout, pubescent like the stem, 5–10 mm. long; leaf-blades lanceolate, subcoriaceous, 7–10 cm. long, 2–3 cm. wide, acuminate, entire, rounded at base, 3-pli-nerved with an additional pair of marginal veins, the principal laterals arising 1–2 mm. above the base and outwardly curved, the straight secondaries 2–3 mm. apart, ascending at an angle of about 60°, obscure above; primaries lightly impressed above; tertiaries obscure; panicle nearly sessile, 6–8 cm. long, sparsely branched, tomentulose like the stem; bracts minute, ovate; flowers 5-merous, sessile and somewhat secund; hypanthium campanulate, 3.2 mm. long to the torus, closely and minutely stellate-pubescent; calyx-tube somewhat spreading, prolonged 0.6 mm. to acute sinuses, minutely pubescent between the sepals within; sepals spreading, triangular, 0.5 mm. long, acute; exterior teeth soft, spreading, subulate, 0.4 mm. long; petals reflexed at anthesis, elliptic-oblong, 6.4 mm. long, 2.5 mm. wide, obliquely retuse, conspicuously glandular-ciliate; filaments slender, glabrous, 5 or 3.2 mm. long; anthers subulate, arcuate, 4.3 or 3.5 mm. long, 2-celled, opening by a small terminal pore; connective gradually broadened and thickened below, 3-lobed at base; ovary 3-celled, half-inferior, ellipsoid, the summit glabrous; style slender, 10 mm. long, very sparsely and minutely glandular-puberulent; stigma truncate.

Type, *Buchtien 999a,* collected at San Carlos, in the Mapiri Region, Bolivia, alt. 850 m., and deposited in the herbarium of The New York Botanical Garden. In the larger episepalous stamens the lateral and dorsal lobes of the connective are approximately equal in size; in the epipetalous stamens the lateral lobes are broadly rounded and much larger than the minute dorsal lobe. In the size, shape, and glandulosity of its petals and the peculiar pubescence of the calyx-tube, *M. pubicalyx* is sharply differentiated from previously known species of the sections *Jucundæ* and *Tamonea.*

Miconia obliqua sp. nov. § *Tamonea:* ramis obtuse 4-angulatis et leviter sulcatis tenuiter stellato-furfuraceis ad nodos valde incrassatis appendicibus stipuliformibus late rotundatis suffultis; petiolis elongatis tomentellis; laminis amplis ovatis acuminatis integris basi cordatis 7–9-nerviis supra pilis purpurascentibus dense setosis subtus ad venas furfuraceis ad venulas glandulosopunctatis; panicula ampla multiflora ramosa, axibus acute 4-angulatis stellato-tomentellis ad nodos incrassatis; floribus 5-meris sessilibus vel breviter pedicellatis; hypanthio tubuloso-campanulato furfuraceo; sepalis brevibus rotundatis, dentibus exterioribus minutis; petalis oblongis late et oblique retusis; antheris imparibus isomorphis subulatis arcuatis; connectivo basi breviter producto subtruncato sub-3-lobato; ovario 5-loculare semi-infero; stylo gracile elongato; stigmate truncato.

Upper branches prominently 4-angled and shallowly sulcate, thinly stellate-furfuraceous, the nodes greatly swollen and bearing a pair of broadly rounded,

stipuliform appendages, the latter gradually reduced in size distally; leaves slightly unequal in the single pair available; petioles 5 or 8 cm. long, stellate-tomentulose; blades membranous, ovate, 15 or 22 cm. long, 11 or 12 cm. wide, acuminate, entire, cordate at base, 7–9-nerved; upper surface freely setose with purplish hairs 2–3 mm. long; lower surface thinly furfuraceous on the primaries and secondaries, punctate with shining particles on the tertiaries, glabrous elsewhere; primaries and secondaries plane above, lightly elevated beneath, the latter about 5 mm. apart, arising at nearly right angles; tertiaries obsolete above, plane and finely reticulate beneath; panicle freely branched, 25 cm. long, its branches sharply 4-angled, stellate-tomentulose; flowers 5-merous, sessile or short-pedicelled; hypanthium tubular-campanulate, 3.3 mm. long to the torus, minutely and thinly furfuraceous toward the base; calyx-tube prolonged 0.5 mm. to rounded sinuses; sepals depressed-rotund, 0.3 mm. long; exterior teeth reduced to minute adnate thickenings; petals oblong, 3.5 mm. long, thinly furfuraceous externally, very broadly and obliquely retuse, leaving an acute apex on one side; stamens isomorphic but slightly unequal; filaments slender, slightly flattened, 4.3 or 3.7 mm. long; anthers subulate, arcuate, 2-celled, opening by a minute terminal pore, 4 or 3.1 mm. long; connective dilated below, prolonged 0.4 mm. into a tubular, obscurely 3-lobed structure surrounding the summit of the filament; ovary half-inferior, 5-celled, its free summit glabrous, truncate-conic; style slender, 7.7 mm. long, glabrous; stigma truncate, 0.3 mm. in diameter.

Type, *Buchtien 1083,* collected at Pararani, in the Mapiri region of Bolivia, alt. 1000 m., and deposited in the United States National Herbarium, no. 1399428. *M. obliqua* differs from previously described species of its section in the very large appendages at the nodes; the shape of its petals is also noteworthy.

Miconia cretacea sp. nov. § *Eumiconia Glomeratiflorae:* ramis, petiolis, et foliorum pagina inferiore indumento cretaceo albo dense obtectis; foliis breviter petiolatis; laminis ellipticis utrinque acutis integris 3-nerviis vel sub-3-pli-nerviis supra glabris; panicula ampla ramosa axibus dense stellato-tomentosis; floribus sessilibus glomeratis 5-meris; hypanthio obconico stellato-tomentoso; sepalis rotundatis brevibus; petalis oblongis extus stellatis; staminibus isomorphis; filamentis gracilibus glabris; antheris linearibus, connectivo simplice; ovario 2-loculare semi-infero, summo minute puberulente; stylo elongato; stigmate capitato.

Stems, petioles and lower leaf-surface densely covered with a white, chalky, detergible indument; petioles stout, angular, 1–2 cm. long; leaf-blades firm, elliptic, 12–23 cm. long by half as wide, acute at both ends, 3-nerved with an additional pair of marginal veins, smooth and somewhat shining above; veins plane above, lightly elevated beneath, the secondaries arising at right angles to the midvein, about 5 mm. apart; panicle ample, freely branched, 13 cm. long, densely stellate-tomentose; flowers sessile in rounded glomerules, 5-merous; hypanthium obconic, 3 mm. long to the torus, densely gray-stellate-tomentose; calyx-tube prolonged 0.6 mm. to rounded sinuses, pubescent like the hypanthium; sepals depressed-rotund, about 0.3 mm. long; exterior teeth minute adnate thickenings; petals oblong, 3 mm. long, 1.4 mm. wide, densely white-stellate externally; stamens isomorphic but variable in size; filaments slender, 2.6–3.4 mm. long; anthers linear, nearly straight, 3.2–3.9 mm. long, the connective neither appendaged nor prolonged; ovary half-inferior, 2-celled,

the free summit minutely puberulent; style slender, 7.7 mm. long; stigma capitate.

Type, *Buchtien 1025,* collected at Mapiri, Bolivia, alt. 550 m., and deposited in the United States National Herbarium, no. 1399438. *Buchtien 1026,* from the same locality, is identical but sterile. It is closely related to the Peruvian *M. crassipes* Triana, in which the leaves tend to be rounder or even subcordate at base, the flowers are smaller, and the indument of the leaves merely tomentose. The indument alone is sufficient to distinguish *M. cretacea,* it being nearly as white as the paper to which the specimen is attached.

Miconia Herrerae sp. nov. § *Glossocentrum:* ramis crassis paullum complanatis stellato-tomentellis; petiolis brevibus; laminis subcoriaceis ellipticis utrinque subacutis integris 3-nerviis supra opacis glabris venis impressis, subtus stellato-pubescentibus venis venulisque elevatis reticulatis; panicula ampla ramosa axibus stellato-tomentosis; floribus 5–8-(saepissime 6-)floris sessilibus arcte glomeratis; hypanthio turbinato-campanulato, tenuiter stellato-pubescente; sepalis brevibus late rotundatis e sinubus latis, dentibus exterioribus minutis; petalis obovato-oblongis; staminibus circa 20; filamentis gracilibus directis vel sub apicem geniculatis; antheris oblongis obtusis poro lato dehiscentibus; connectivo basi in lobos 4 brevissimos producto; ovario 4-loculare; stigmate capitato.

Flowering branches stout and simple, slightly compressed, thinly stellate-tomentulose when young, glabrate in age, the internodes 4–6 cm. long; petioles stout, 15–20 mm. long, finely stellate-pubescent; blades rather thick and firm, elliptic, as much as 18 cm. long by half as wide, subacute at both ends, entire, 3-nerved with an additional pair of submarginal veins, densely stellate-pubescent above when young, soon becoming glabrous, loosely and permanently stellate-pubescent beneath; veins all impressed lightly above, the secondaries 3–6 mm. apart, ascending at an angle of about 80°; veins all strongly elevated and conspicuously reticulate beneath; panicle 14 cm. long, its branches closely but thinly stellate-tomentulose; flowers 5–8-merous, usually 6-merous, sessile, densely aggregated in small glomerules; hypanthium turbinate-campanulate, 2.7 mm. long to the torus, thinly stellate-pubescent; calyx-tube prolonged about 0.6 mm. to broad flat sinuses; sepals depressed-rotund, membranous, 0.3 mm. long, the external teeth minute flat subapical thickenings; petals broadly obovate-oblong, 2.4 mm. long, glabrous, equilateral, obscurely retuse; stamens about 20; filaments slender, glabrous, 2.3 mm. long, those of the episepalous series and 1 or 2 others straight, the remainder geniculate near the summit; anthers stoutly oblong, 2 mm. long, opening by a wide terminal pore, 2-celled; connective scarcely elevated but broadened toward the base and prolonged on each side into 2 very short obtuse basal lobes; ovary inferior, 4-celled with numerous ovules, its summit depressed-rotund, glabrous; style slender, glabrous, 5 mm. long; stigma capitate, 0.6 mm. in diameter.

Type, *Herrera 1575,* collected at Cedrobamba in the Urubamba Valley, Peru, alt. 2200 m., and deposited in the United States National Herbarium, no. 1342355. In a series of 32 flowers, 3 were 5-merous, 23 6-merous, 5 7-merous, and 1 8-merous; in three flowers the stamens numbered 20, 21, and 22. Of these, the six episepalous stamens and one or two others, inserted above the middle of the petals, had straight filaments, the remainder had

geniculate filaments and were inserted two or three above each petal. Their geniculation gives them the aspect of § *Cremanium,* while their shape and the glomerate flowers suggests § *Glossocentrum,* where the species almost certainly belongs. Several species with the general habit of our plant grow in southern Brazil, most of them with densely tomentose or pli-nerved leaves, and none of them, so far as known to me, with more than the normal two circles of stamens. One polyandrous species of the section, *M. icosandra* Gl., has recently been collected in Peru by Killip & Smith, but ours differs in its stellate pubescence on stem, foliage, inflorescence, and hypanthium and in the large number of ovules in each loculus of the ovary.

Miconia pterogona sp. nov. § *Amblyarrhena;* caulibus 4-alatis, superne lepidota furfuracea; foliis brevissime petiolatis chartaceis obovato-oblongis abrupte acutis integris basi subrotundatis 3-pli-nerviis, venis marginalibus 2 adjectis, ad venas minutissime furfuraceis ceterum utrinque glabris; paniculis parvis confertis; floribus sessilibus 5-meris; hypanthio subgloboso tenuissime tomentello; sepalis late triangularibus; petalis late oblongis; staminibus isomorphis; filamentis glabris superne geniculatis; antheris oblongis, connectivo simplice; ovario semi-infero 3-loculare; stylo glabro, stigmate truncato.

A low tree, the stems sharply 4-winged, lepidote-furfuraceous above, becoming glabrous below; petioles stout, 5 mm. long, furfuraceous like the stem; blades firm, obovate-oblong, as much as 15 cm. long by 6 cm. wide, abruptly acute, entire, narrowed from below the middle to a subrotund base, 3-pli-nerved, with an additional pair of marginal veins, minutely arachnoid-furfuraceous on the veins beneath, otherwise glabrous; primaries impressed above, elevated beneath; secondaries plane above, slightly elevated beneath, 3–5 mm. apart, curved-ascending at an angle of about 70°; tertiaries plane on both sides, very finely reticulate, the areoles 0.5 mm. wide or less; panicle crowded, about 7 cm. long, its branches 4-winged, thinly gray-tomentulose; flowers 5-merous, sessile, subtended by oblong to obovate bracts 2–3 mm. long; hypanthium subglobose, 2.3 mm. long to the torus, very thinly arachnoid-tomentulose; calyx-tube erect, prolonged 0.3 mm. to broadly rounded sinuses; sepals broadly triangular with concave sides, 0.5 mm. long, acute, the exterior teeth not differentiated; petals broadly oblong-ovate, 1.8 mm. long, nearly equilateral, minutely retuse; stamens isomorphic; filaments stout, flat, 2.7 mm. long, geniculate 0.7 mm. below the summit; anthers oblong, 1.9 mm. long, conspicuously bent outwardly near the tip to a dorso-terminal pore; connective elevated into a conspicuous ridge, neither appendaged nor prolonged; ovary 3-celled, half-inferior, its free summit sharply angled and minutely 10-toothed; style glabrous, 4.5 mm. long; stigma truncate.

Type, *Sawada 79,* collected at Pan de Azucar, Huanuco, Peru, and deposited in the herbarium of the Field Museum, no. 607946. The four-winged stems distinguish this species from most members of the section *Amblyarrhena* and, with the short petioles and general habit, indicate its probable affinity to *M. glutinosa* Cogn. The latter differs from ours in its smaller leaves, broadly rounded at the summit and densely tomentose beneath.

Tococa carolensis sp. nov. § *Hypophysca:* frutex bimetralis; caule subterete dense breviterque hirsuto; petiolis brevibus hirsutis; formicariis semiovoideis dense hirsutis; laminis ellipticis acuminatis basi acutatis 3-nerviis

utrinque setosis; inflorescentia axillare subumbellata pauciflora conferta, pedicellis brevissimis; hypanthio campanulato densissime hirsuto parietibus crassis; sepalis ovatis obtusis, dentibus exterioribus subulatis tota longitudine adnatis setosis; petalis anguste obovatis; antheris linearibus poro ventro-terminale dehiscentibus, connectivo dorso elevato basi minute calloso; ovario infero summo glabro; stylo elongato glabro, stigmate subgloboso.

Stem shrubby, about 2 m. tall, essentially terete throughout, densely hirsute with stout, erect, partly glandular hairs about 2 mm. long, underlaid by a dense indument of minute glandular hairs, the internodes about 10 cm. long; leaves conspicuously dimorphic; small leaves: formicaria none or rudimentary; petioles about 10 mm. long, pubescent like the stem; blades elliptic, 7–11 cm. long, 4–5.5 cm. wide, abruptly short-acuminate or cuspidate, obtuse at base, otherwise like the larger; large leaves: free petiole 6–8 mm. long, pubescent like the stem, the hairs often 3 mm. long; formicaria petiolar, semi-ovoid, 12–18 mm. long, densely hirsute and minutely glandular; blades thin, bright green, elliptic, 15–20 cm. long and nearly half as wide, acuminate to a slender cusp 1 cm. long, entire, conspicuously ciliate, broadly acute at base, 3-nerved with an additional pair of weak, undulate submarginal veins; primaries impressed above, elevated beneath; secondaries obscure above, lightly elevated beneath, 5–8 mm. apart, nearly straight, arising at an angle of 80–90°; tertiaries nearly obsolete above, plane and reticulate beneath; upper surface setose with erect simple hairs 2–2.5 mm. long, 1–1.5 mm. apart; lower side conspicuously setose on the primaries with hairs about 2 mm. long, sparsely setose elsewhere with somewhat shorter hairs; inflorescence axillary, few-flowered, densely subumbellate, its short axes densely setose and substellate, bracts subulate, 2 mm. long; flowers 5-merous, nearly sessile; hypanthium campanulate, 6 mm. long to the torus, with very thick and leathery walls, densely hirsute with slender hairs 2–3 mm. long, about 0.3 mm. apart, with a minute terminal gland, and freely glandular-puberulent beneath them; calyx-tube slightly spreading, prolonged 1.2 mm. to rounded sinuses, pubescent like the hypanthium; sepals broadly ovate, obtuse, 1.8 mm. long, membranous, sparsely hirsute and minutely glandular; exterior teeth subulate, hirsute, adnate to the sepals to their tips and thence continued into setae projecting 2 mm.; petals narrowly obovate, 10.5 mm. long, 7 mm. wide, slightly inequilateral, obtuse; filaments 6 mm. long, flattened, 0.8 mm. wide, glabrous; anthers stoutly linear, 4 mm. long, obtuse, 2-celled, with convoluted thecae, opening by a ventro-terminal pore; connective somewhat elevated into a narrow dorsal ridge, bearing two minute callosities at base; ovary almost wholly inferior, the glabrous summit prolonged nearly 1 mm. into an annular thickening around the style; style stout, glabrous, 9 mm. long; stigma subglobose, 2 mm. in diameter.

Type, *Buchtien 976,* collected at San Carlos, near Mapiri, Dept. La Paz, Bolivia, and deposited in the herbarium of The New York Botanical Garden; *Buchtien 977* and *Bang* without number are the same. In the flower dissected the ovary was 6-celled and the stamens 13, an anomaly which is probably not often found. The distinctive feature of the species is the small, subumbellate, axillary inflorescence, a single stem usually bearing two such clusters at adjacent nodes. In this it agrees with *T. juruensis* Pilger, in which the stems are sulcate, covered with longer and paler bristles, the free petiole longer, the formicaria larger and less densely hirsute, the leaf-blades

larger, proportionately wider, and much less hirsute, and the sepals conspicuously ciliate.

Clidemia calcarata Cogn. & Gl. sp. nov. § *Sagraea:* ramis pubescentibus pilis flexuosis brunneis, ramis gracillimis; petiolis brevibus dense pubescentibus; laminis parvis membranaceis ovatis acutis minutissime denticulatis basi rotundatis vel subcordatis 5-pli-nerviis, supra glabris aeneis venis venulisque impressis, subtus praeter venas pubescentes glabris; panicula laxa ramosa, axibus gracilibus furfuraceis, bracteis setaceis; floribus 4-meris breviter pedicellatis; hypanthio campanulato minutissime furfuraceo ad mediam leviter constricto; sepalis late rotundatis membranaceis, sinubus latis, dentibus exterioribus teretibus sepala paullum excedentibus; petalis obovatis; filamentis complanatis; antheris oblongis 2-locularibus poro terminale dehiscentibus, connectivo deorsum in calcarem subulatum producto; ovario 4-loculare; stylo gracile, stigmate capitato.

Stems woody, slender, sparingly branched, densely pubescent with crooked, more or less incurved, brownish hairs; petioles slender, 5–10 mm. long, densely pubescent; leaf-blades thin, ovate, as much as 55 by 30 mm., acute, minutely denticulate, rounded or subcordate at base, 5-pli-nerved, the larger with an additional pair of submarginals; upper side glabrous, bronze-green, with prominently impressed veins; lower side gray-green, densely pubescent on the primaries, elsewhere glabrous; panicle lax, sometimes nodding, 5–7 cm. long, its axes very slender, thinly furfuraceous; bracts setaceous, 1–2 mm. long; pedicels about 1 mm. long; hypanthium campanulate, 2.5 mm. long to the torus, constricted at the summit of the ovary, very closely and minutely furfuraceous; calyx-tube prolonged 0.3 mm. to broad flat sinuses; sepals broadly rounded, membranous, 0.3 mm. long from the sinuses; exterior teeth erect, terete, blunt, barely surpassing the sepals; petals obovate, 4 mm. long, slightly inequilateral and minutely retuse; stamens 8, isomorphic; filaments flat, glabrous, 2.2 mm. long; anthers 1.6 mm. long, stout and blunt, opening by a minute terminal pore, 2-celled, the connective slightly elevated on the back and prolonged at base into a subulate spur 0.5 mm. long; ovary wholly inferior, 4-celled; style slender, glabrous, 6 mm. long; stigma capitate.

Type, *Lehmann 5472,* collected on the western slopes of the west Andes of Popayán, Colombia, alt. 1600–3000 m., and deposited in the United States National Herbarium, no. 1420328. On a sheet of the same number in the herbarium at Berlin Cogniaux has written the species-name adopted here; he did not publish a description. In general habit and floral structure the plant suggests a *Miconia* of the section *Amblyarrhena.* The panicles of our plant, while partly terminal like those of *Miconia,* soon become lateral by proliferation of the branches, a character frequently seen in *Clidemia.* The anthers of our plant are 2-celled, while those of *Amblyarrhena* are 4-celled. Its relationship within the genus *Clidemia* is problematical.

Clidemia fissinervia sp. nov. § *Sagraea:* frutex metralis, caule subsimplice sursum companato minute pulverulento; foliis in quoque jugo subimparibus; petiolis elongatis supra dense pilosis; laminis amplis lanceolatis acuminatis subintegris basi acutis vel cuneatis 3-nerviis, supra subglabris, subtus ad venam mediam pilosis ceterum minute resinosis; inflorescentia axillare parva bracteis minutis subulatis; floribus 4-meris subsessilibus; hypanthio tubuloso pulverulento; sepalis erectis ovatis, dentibus exterioribus

patulis conicis; petalis obovato-oblongis; antheris lanceolatis; ovario 4-loculare; stylo elongato stigmate minuto subgloboso.

Stems shrubby, 1 m. high, conspicuously flattened above, minutely and thinly pulverulent, the internodes 2–3 cm. long; leaves somewhat unequal in each pair; petioles slender, 2–4.5 cm. long, thinly pulverulent below, densely pilose above with curved hairs 0.8 mm. long; blades thin, bright green, scarcely paler beneath, lanceolate, 9–18 cm. long, 3.5–5 cm. wide, long-acuminate and somewhat falcate at tip, entire or very minutely denticulate, obscurely and evanescently ciliate, acute or cuneate at base, 3-nerved from the base, the lateral primaries emitting each a submarginal vein shortly above the base; primaries and secondaries impressed above, elevated beneath, the latter 3–5 mm. apart, arising at nearly right angles with the midvein; tertiaries obscure, impressed on both sides, very finely reticulate; upper side furfuraceous on the midvein, very minutely puberulent on the surface; lower side minutely resinous-pulverulent, sparsely pilose along the midvein; inflorescence axillary, small and few-flowered; bracts subulate, 0.5 mm. long; flowers 4-merous, on very short pedicels; hypanthium tubular, slightly swollen at base, 3.6 mm. long to the torus, obscurely ribbed, finely gray-pulverulent; calyx-tube erect, prolonged 0.2 mm. to rounded sinuses; sepals membranous erect, ovate, broadly obtuse, the free tip 0.2 mm. long; exterior teeth spreading, stoutly conic, 0.6 mm. long, pulverulent like the hypanthium; petals recurved, obovate-oblong from a broad base, 1.5 mm. long, 1 mm. wide, broadly rounded or subtruncate above; stamens isomorphic, erect at anthesis; filaments stout, somewhat flattened, 1.6 mm. long; anthers oblong, slightly arcuate, 1.7–1.8 mm. long, laterally flattened, opening by a minute terminal pore, 2-celled; connective neither elevated, appendaged, nor prolonged; ovary wholly inferior, 4-celled; style slender, 5.6 mm. long, slightly curved, gradually tapering; stigma not expanded, 0.2 mm. in diameter, hemispherical.

Type, *Buchtien 5496,* collected at Hacienda Simaco, Bolivia, alt. 1400 m., and deposited in the herbarium of The New York Botanical Garden; *Buchtien 7401* and *Tate 1086* are identical. The species is closely related to *C. obliqua* (Triana) Cogn. and *C. acutifolia* Cogn., but is distinguished by its larger, proportionately narrower, 3-nerved leaves pilose along the midvein beneath.

Clidemia laevifolia sp. nov. Caulibus hirsutis et minute stellatis; laminis ovatis vel ovato-oblongis acuminatis integris ciliatis basi subrotundatis 3-pli-nerviis supra glabris venis impressis subtus ad paginam glabris ad venas hirsutis; inflorescentia parva trichotoma; floribus 5-meris breviter pedicellatis; hypanthio campanulato dense hirsuto pilis simplicibus; sepalis undulatis subscariosis, dentibus exterioribus subulatis hirsutis hypanthio paullum brevioribus; toro squamis fimbriatis ornato; petalis oblongo-obovatis; antheris subulatis; ovario in coronam cylindricam breviter 5-lobatam producto.

Stems bristly hirsute with straight simple hairs, underlaid by minute stellate pubescence; petioles slightly unequal, 5–12 mm. long, hirsute like the stem; leaf-blades firm, narrowly ovate or ovate-oblong, 8–11 cm. long, 3.5–4.5 cm. wide, sharply acuminate, entire, conspicuously ciliate with simple hairs, obtuse to subrotund at base, subrevolute, 3-pli-nerved, or with a faint additional pair, upper surface glabrous, the veins prominently impressed and the midvein thinly stellate-furfuraceous near the base, lower surface glabrous, the primaries and occasionally the secondaries hirsute; inflorescence trichotomously branched, 1–2 cm. long, pubescent like the stem but with more

abundant stellate hairs; pedicels 1 mm. long; flowers 5-merous; hypanthium campanulate, 5.2 mm. long to the sinuses, rather acute at base, neither flaring nor constricted, densely hirsute with long, simple, spreading hairs; calyx-tube prolonged 0.5 mm., scarious; sepals reduced to undulate projections 0.4 mm. long, the free margin 0.1–0.2 mm. wide, the exterior teeth subulate, hirsute, 3–3.7 mm. long; torus with 10 flat, penicillate-margined scales; petals somewhat obliquely oblong-obovate, 10 mm. long, 4.4 mm. wide, rounded at apex, many-nerved; filaments slender, 3 mm. long; anthers subulate, nearly straight, 3.5 mm. long; ovary tipped with a cylindric neck terminating in 5 shallow glabrous lobes; style 9 mm. long.

Type, *Wachenheim 127,* collected at Godebert, French Guiana, March, 1920, and deposited in the herbarium of The New York Botanical Garden. Its close relation to *Clidemia hirta* (L.) D. Don is shown by numerous characters, most important of which are the receptacular scales. It differs from that species in its pli-nerved leaves which are essentially or completely glabrous on the surface, proportionately narrower, more acute at base, and with strongly impressed veins, less pubescence along the midvein, longer ciliation, and shorter petioles, and in its much narrower petals.

Clidemia rigida Cogn. et Gl. sp. nov. § *Staphidium:* caulibus fruticosis crassis longe denseque hirsutis; petiolis elongatis hirsutis; laminis crassis ovatis acutis integris basi cordatis 7-nerviis supra glabris subtus hirsutis; cymis parvis hirsutis; floribus bracteatis breviter pedicellatis 5-meris; hypanthio subgloboso hirsuto; sepalis magnis ovatis dentes exteriores triangulares adnatos excedentibus; petalis concavis rotundis; connectivo basi incrassato breviter producto; ovario 3-loculare; stigmate truncato.

Stems stout, densely hirsute with dark purple hairs 3–4 mm. long; petioles stout, 2–3 cm. long, hirsute like the stem; blades subcoriaceous, ovate, 7–11 cm. long by three-fifths as wide, acute, entire, cordate at base, 7-nerved, glabrous above with strongly impressed veins, beneath pubescent with crooked hairs, the veins elevated and conspicuously reticulate; cymes axillary, few-flowered, 10–15 mm. long, densely hirsute; flowers 5-merous, on short hirsute pedicels subtended by linear bracts 1–2 mm. long; hypanthium subglobose, 2.2 mm. long to the torus, densely hirsute with stout purple hairs 2–3 mm. long, underlaid by a thin and obscure stellate indument; calyx-tube hirsute, prolonged 0.8 mm. to very narrow sinuses; sepals broadly depressed-ovate, 1.3 mm. long, glabrous; exterior teeth triangular, acute, almost wholly adnate, hirsute, shorter than the sepals; petals concave, rotund, 5 mm. long, retuse, glabrous; stamens isomorphic; anthers very stout and blunt, 2.3 mm. long, the connective thickened basally into a stout conic organ prolonged 0.3 mm. below the thecae to a truncate base; ovary 3-celled; stigma truncate.

Type, *Lehmann 6132,* without data, from Colombia or Ecuador, deposited in the United States National Herbarium, no. 939385. Although recognized as new and named by Cogniaux many years ago, this species has remained undescribed. According to the arrangement of species in the *Monograph,* it is related to numbers 26 to 33, but differs from all of them in its leaves glabrous above and from each of them in various details of size, form, or structure. It probably stands closest to *C. capitellata* (Bonpl.) Don, which is at once distinguished by its spicate flowers.

Ossaea Buchtieni sp. nov. Frutex ramosus; ramis gracilibus densissime breviterque pubescentibus; petiolis brevibus dense pubescentibus; laminis oblongo-lanceolatis subacuminatis basi obtusis margine minute spinuloso-denticulatis 5-nerviis supra dense breviterque papilloso-pubescentibus subtus molliter villosis; inflorescentia parva laxa pauciflora ex axillis superioribus foliorum tertiam partem aequante, axibus villosis, bracteis setaceis; floribus 5-meris in glomerulis 2–3-floris sessilibus; hypanthio subgloboso densissime albo-villoso; sepalis triangularibus lateribus concavis e sinubus rotundatis; petalis subulatis dorso villosis, dente dorsale apicem inflexum paullum excedente; antheris linearibus obtusis, connectivo simplice; ovario semi-infero 3-loculare, summo conico minute sericeo; stigmate punctiforme.

Branching shrub 1.5 m. high; branches slender, very densely pubescent with curved-ascending, reddish-brown hairs about 0.5 mm. long, the internodes 1–3 cm. long; petioles pubescent like the stem, 5–8 mm. long; blades rather firm, oblong-lanceolate, 7–10 cm. long, 22–32 mm. wide, subacuminate to an acute tip, inconspicuously spinulose-denticulate, broadly cuneate or obtuse at base, 5-nerved; upper surface deep green, strigose on the primaries, densely papillose-pubescent on the surface with curved-ascending hairs about 0.8 mm. long; lower surface softly villous throughout with white, nearly erect hairs about 1 mm. long, those of the primaries somewhat shorter and stouter; only the midvein prominent above, all veins prominent and lightly elevated beneath, the secondaries about 2 mm. apart; inflorescence from the upper axils, spreading or nutant, 2–4 cm. long, bearing 2 pairs of short lateral branches, each and the central axis with a terminal glomerule of 2–3 sessile, 5-merous flowers, the axes densely gray-villous; bracts setaceous, villous, 3 mm. long; hypanthium green, subglobose, 2 mm. long to the torus, densely white-villous with simple hairs; calyx-tube prolonged about 0.2 mm. to broadly rounded sinuses; sepals triangular with concave sides, 0.6 mm. long, acute, densely villous, the exterior teeth not differentiated; petals lanceolate-subulate, 2.1 mm. long, densely villous on the back, the actual apex slightly inflexed and exceeded ⅓ mm. by the erect dorsal tooth; anthers linear, blunt, the connective entirely simple; ovary half-inferior, 3-celled, the free summit conic, densely sericeous; stigma punctiform.

Type, *Buchtien 986,* collected at San Carlos, Bolivia, alt. 850 m., and deposited in the herbarium of The New York Botanical Garden. The filaments are as yet not elongated; the anthers, probably nearly or quite full-sized, are 1.2 mm. long; the style, as yet only 1.5 mm. long, is glabrous and terete. It is a member of the section Glaziophytum, in which I know of no other species with which it may well be compared.

Dicranostyles sericea sp. nov. Frutex scandens; ramis subteretibus glabris; petiolis tenuiter pubescentibus; laminis oblongo-ellipticis basi acutis apice subiter angustatis et acuminatis subtus fulvis tenuiter sericeis; racemis pluribus axillaribus fulvo-sericeis, bracteis minutis sericeis ovato-triangularibus, pedicellis brevibus; calycis lobis late rotundatis obtusis bene imbricatis extus sericeis; corolla campanulata extus dense sericea infra medium 5-fida, lobis oblongis apice inflexis; staminibus corollae tubo insertis; filamentis infra medium late complanatis et dense glanduloso-hirtellis, superne angustatis glabris; antheris ovatis basi alte cordatis; stylo subintegro, infra apicem brevissime 2-fido; stigmate subgloboso.

A climbing woody vine, the flowering branches slender, subterete, glabrous; petioles slender, 15–20 mm. long, minutely pubescent; blades firm, oblong-elliptic, 11–14 cm. long, 5–6 cm. wide, broadly acute at base, entire, abruptly acuminate into a narrowly triangular tip 1–2 cm. long, glabrous above, thinly fulvo-sericeous beneath; veins impressed above, prominent beneath, the secondaries about 12 on each side, ascending, with numerous intermediates, the tertiaries inconspicuous above, reticulate beneath; inflorescence of several (3–6) racemes from the upper axils, each 3–5 cm. long, with numerous nearly sessile flowers, the axes strongly angular, densely brown-sericeous; bractlets ovate-triangular, about 1 mm. long, sericeous; calyx imbricate, the sepals coriaceous, rotund, 1.5 mm. long, obtuse or barely apiculate, densely sericeous without; corolla campanulate, 4 mm. long, its tube glabrous, 1.3 mm. long, its lobes narrowly oblong, 2.7 mm. long, densely sericeous without, inflexed at the summit; stamens inserted on the corolla-tube; filaments nearly 2 mm. long, nearly 0.5 mm. wide at base, narrowed above, densely glandular-pubescent just below the middle; anthers cordate-ovate, 1.3 mm. long, obtuse, deeply cordate at base; ovary semi-ellipsoid; style 0.5 mm. long, almost entire, the subglobose stigmas closely connivent.

Type, *Klug 1132,* collected at Mishuyacu, near Iquitos, Dept. Loreto, Peru, and deposited in the herbarium of The New York Botanical Garden. Six species of this small genus of Convolvulaceae have hitherto been described, of which four have the plainly divided style of the type. The other two somewhat aberrant species, with style entire or nearly so, are *D. holostyla* Huber and *D. Mildebraediana* Pilger. The former, from Pará, differs notably from ours in its narrowly mucronulate leaves whitish beneath, long pedicels, nearly glabrous calyx, and corolla lobed only above the middle. The latter, also from eastern Peru, stands much nearer to *D. sericea,* but differs in its glabrous leaves, its pilose rather than sericeous inflorescence, and its smaller and merely pilose corolla.

Stilpnopappus Pittieri sp. nov. Caule erecto gracile angulato paullum lanato inferne foliato; petiolis brevibus lanatis; laminis anguste obovatis acutis subintegris basi cuneatis supra tenuiter pubescentibus subtus cano-tomentosis; pedunculis terminalibus solitariis elongatis nudis; capitulis 2–3 subsessilibus circa 25-floris; involucro late hemisphaerico villoso, squamis exterioribus brevioribus ovato-lanceolatis interioribus longioribus anguste lanceolatis acuminatis; corollis glabris lobis lanceolatis; antheris subsagittatis; achaeniis obovoideis villosis; pappi squamis exterioribus circa 10 ovatis erosis, interioribus 10–12 linearibus.

Stems slender, erect, simple, 3–5 dm. tall, prominently angled, thinly lanate; leaves on the basal third or half of the plant only; petioles densely lanate, 2–4 mm. long; blades thin, narrowly obovate, 30–45 mm. long, 10–15 mm. wide, acute, nearly entire, cuneate from above the middle to the base, dark green and thinly pubescent above, the hairs about 0.7 mm. long, densely canescent-tomentose beneath, the veins obscure above and mostly concealed beneath; peduncles solitary, terminal, leafless, 25–35 cm. long, thinly lanate like the stem; heads 2 or 3, terminal and subterminal, sessile or subsessile, densely lanate at the base, about 25-flowered; involucre broadly hemispheric or turbinate, densely villous with hairs about 1 mm. long, outer scales ovate-lanceolate, 2 mm. long, acute, the inner narrowly lanceolate, 4.5 mm. long,

acuminate; receptacle convex, deeply alveolate with lacerate margins; corollas purple, 5 mm. long, glabrous, the lobes lanceolate, 1.1 mm. long; anthers 1 mm. long, of which the triangular appendage constitutes a third, barely sagittate at base; style-branches 0.8 mm. long, pubescent externally; achenes turbinate or obovoid, obscurely 10-ribbed, 1.1 mm. long, 0.8 mm. in diameter, slightly constricted at the summit, densely villous with spreading hairs 0.8 mm. long; outer pappus scales about 10, ovate-elliptic, 1.7 mm. long, 0.7 mm. wide, scarious, imbricate, erose-margined; inner pappus-scales about 10–12, scarious with a thickened midnerve, linear-lanceolate, 3.7 mm. long, 0.3 mm. wide, erosely toothed above the middle.

Type, *Pittier 12,491*, collected at the margin of a savanna, Mesa de El Sombrero, Dept. Guarico, Venezuela, 10 Sept. 1927, and deposited in the United States National Herbarium, no. 1,344,033. Its extraordinarily long-peduncled inflorescence and thin obovate leaves differentiate it from the other species of the genus.

Vernonia Rusbyi nom. nov.

Vernonia arborescens cuneifolia Britt., Bull. Torrey Club 18: 331. 1891.

Vernonia cuneifolia Gleason, Amer. Jour. Bot. 10: 301. 1923. Not *Vernonia cuneifolia* Gardn., Hook. Lond. Jour. Bot. 5: 215. 1846.

Vernonia tovarensis sp. nov. *Lepidaploa Scorpioideae Foliatae:* caule fruticoso submetrale simplice cinereo-tomentoso; petiolis crassis brevibus tomentosis; laminis ovato-lanceolatis acuminatis integris basi obtusis supra scabridis subtus tomentosis; racemis axillaribus paniculatis superioribus longioribus, bracteis ovato-lanceolatis; capitulis in quaque axilla 1–3 circa 11-floris; involucro turbinato, squamis laxe imbricatis acuminatis, exterioribus ovatis lanatis, interioribus lineari-lanceolatis apice lanatis ceterum glabris; corollae glabrae lobis acutis; antheris linearibus; achaeniis anguste prismaticis 10-angulatis breviter pilosis; pappi squamis exterioribus linearibus, quam setis interioribus ca. 30 stramineis 7-plo brevioribus.

Suffruticose, about one meter tall; stems stout, apparently simple to the inflorescence, densely but closely cinereous-tomentose; petioles stout, 1 cm. long, densely cinereous-tomentose; leaf-blades firm, ovate-lanceolate, usually about 10 cm. long by 5 cm. wide, the largest as much as 14 cm. long, the upper considerably reduced, acuminate, obtuse at base, entire, scabrously pubescent above with vertical hairs 0.3 mm. long, set 0.1–0.2 mm. apart, densely cinereous-tomentose beneath; lateral veins 10–12 pairs, ascending at angles of 60–70°, obscure above, prominent beneath; inflorescences of several axillary racemes, forming a loose leafy panicle, the upper ones as much as 10 cm. long, the lower much shorter, tomentose like the stem; bracteal leaves ovate-lanceolate, short-petioled, 30–5 mm. long, acuminate and mucronate, tomentose like the foliage leaves; heads about 11-flowered, 1–3 sessile in the axils of the bracts; involucre 4 mm. high, turbinate, its scales about 30, loosely and irregularly imbricate in several series, the outer ovate, the inner linear-lanceolate, all acuminate, the outer lanate, the inner glabrous to the floccose-lanate tip; corolla 7 mm. long, glabrous, its linear-lanceolate acute lobes 2 mm. long; anthers linear, briefly sagittate at base, the body 1.8 mm., the lanceolate obtuse tips 0.7 mm. long; style-branches 1.3 mm. long; achenes linear-prismatic, 10-angled, 1.3 mm. long, sparsely short-pilose; outer pappus-

scales linear, acuminate, ciliate, 0.6–0.7 mm. long, 0.1 mm. wide; inner bristles about 30, 4.5–5 mm. long, pale tawny, flattened, 0.05 mm. wide.

Type, *Pittier 12,794*, collected on bushy slopes near Tovar, Merida, Venezuela, alt. 1200 m., 31 Jan. 1928, and deposited in the herbarium of The New York Botanical Garden.

NEW YORK BOTANICAL GARDEN

STUDIES IN ONAGRACEAE VIII. THE SUBGENERA HARTMANNIA AND GAUROPSIS OF THE GENUS *OENOTHERA*. THE GENUS *GAYOPHYTUM*

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In the preparation of this paper it has been my privilege to see material in many herbaria and it is now a great pleasure to express my deep appreciation to those in charge of the various institutions where I was allowed to work, both for their kindness and that of the various members of their staffs. The names of the herbaria from which I cite material, with the abbreviations I shall use, are as follows:

Botanisches Museum at Berlin-Dahlem (Ber),
Boissier Herbarium at Geneva (Boiss),
British Museum at London (BM),
Delessert Herbarium at Geneva (Del),
Royal Botanical Garden at Edinburgh (E),
Gray Herbarium (G),
Royal Botanical Garden at Kew (K),
Missouri Botanical Garden, special material (M),
New York Botanical Garden (NY),
Jardin des Plantes, Paris (Par),
United States National Herbarium (US).

HARTMANNIA

Hartmannia (Spach) Munz, n. subgenus

Hartmannia, as genus, Spach, Hist. Veg. Phan. 4: 370. 1835; Small, Bull. Torrey Bot. Club 23: 180. 1896; Rose, Contr. U. S. Nat. Herb. 8: 328. 1905. As section, Endlicher, Gen. Pl., 1190. 1840. *Xylopleurum*, as genus, Spach, l.c., 378; Raimann, in Engl. & Prantl, Die nat. Pflanzenfam. III, 7: 214. 1893.

Perennial herbs, with leaves subentire to pinnatifid, and the flowers in the axils of the reduced upper leaves. Calyx-tube funnelform, not usually much longer than, if as long as, the calyx-lobes. Corolla white to rose or purplish, yellow in one species. Alternate stamens slightly longer than the others. Stigma with 4 linear lobes. Capsule club-shaped, ribbed or winged, attenuate at base into a sterile pedicel-like portion or truly pedicellate. Seeds and ovules numerous, clustered on slender funiculi in more than 2 rows. Seeds obovoid, not angled.

Type species, *Oenothera rosea* Ait. This subgenus seems to constitute a fairly definite entity, and is most closely related to the subgenus Kneiffia, which has yellow flowers and a more definitely pedicelled clavate capsule. I am including under Hartmannia, *Oe. multicaulis*, even though this species

has yellow flowers; the curved sterile base of the fruit and the habit of growth are more like those of Hartmannia. There has been also some confusion in the case of species belonging to Gauropsis; these species are discussed under that subgenus. As here recognized, Hartmannia ranges from the southwestern United States to Peru and Bolivia.

Key to Species

Flowers yellow or greenish, drying orange-red; petals 3–6 mm. long; capsule arcuate; stems prostrate ..... 1. *Oe. multicaulis*

Flowers white to rose; petals 5–40 mm. long; capsule usually straight.

Buds nodding; lower sterile part of capsule cylindrical and sessile ..... 7. *Oe. speciosa*

Buds erect, except possibly in *Purpusii;* lower sterile part of capsule tapering toward base and narrowed into a pedicel.

Petals rose to reddish violet, 5–10 mm. long; calyx-tube 4–8 mm. long; capsule 3–4 mm. thick (including wings), strigulose, the wings scarcely 1 mm. wide; plants erect, 1–5 dm. tall or shrubby and taller, strigulose in upper parts .... 6. *Oe. rosea*

Petals white to rose, 1–4 cm. long.

Fruit not winged, narrowly obovoid, 4–6 mm. thick, strigose; stems decumbent, appressed-canescent; leaves sinuate-denticulate, oblanceolate. Central Mexico ..... 3. *Oe. Purpusii*

Fruit winged at the angles, the wings 1–3 mm. wide; not all hairs appressed.

Stems decumbent; cauline leaves oblong, 2–7 cm. long, subentire; petals white, 12–18 mm. long; calyx-tube 5–8 mm. long; capsule 8–10 mm. thick. Central Mexico ..... 2. *Oe. deserticola*

Stems decumbent or ascending; cauline leaves usually pinnatifid or dentate; petals pink or white.

Petals 10–15 mm. long; capsule 4–5 mm. thick, the wings 1 mm. wide; calyx-tube 1–2 cm. long; upper parts of plant canescent-strigose. 4. *Oe. Kunthiana*

Petals 20–35 mm. long; capsule 6–8 mm. thick, the wings 2–3 mm. wide; calyx-tube 1 cm. long; upper parts of plant more or less pilose. 5. *Oe. tetraptera*

Treatment of Species

1. Oenothera multicaulis R. & P., Fl. Peruv. 3: 80, t. 317. 1802

Perennial herb, sometimes suffrutescent at base, with several subglabrous to villous, prostrate or procumbent leafy stems 3–30 cm. long, these simple or sometimes few-branched; leaves of basal rosette broadly oblanceolate, the blades 1–5 cm. long, 5–25 mm. wide, obtuse, subglabrous, except for pubescence on margins and veins of lower surface, gradually narrowed into a winged petiole of about same length as blade; cauline leaves oblong-ovate to lance-ovate, subsessile, 5–30 mm. long, obtuse, pubescent on veins and margins, sometimes with scattering hairs on general surface; flowers solitary, sessile, axillary, only one or 2 in anthesis at once, apparently diurnal; calyx-tube 4–8 mm. long, more or less pubescent without, pubescent within at very base, often purplish; calyx-lobes lanceolate, pubescent, 3–6 mm. long, without free tips in bud, distinct in anthesis, green with purplish tinge; petals yellow or greenish, becoming orange red, broadly obovate, 3–6 mm. long; stamens subequal, one-half to two-thirds the length of the petals, the anthers glabrous and equaling or slightly exceeding the glabrous, slightly flattened filaments; style equaling stamens, pubescent at very base;

stigma-lobes ovoid, ca. 1 mm. long; capsule clavate, subglabrous to villous, 1–2 cm. long, arcuate, 1–1.5 mm. thick in lower half, 3–4 mm. thick in upper, somewhat 4-sided and angled or winged, with prominent vein down the middle of each face; seeds numerous, brown, asymmetrically obovoid, 0.5–1.5 mm. long, minutely cellular-pitted, but appearing smooth under the ordinary lens.

KEY TO VARIETIES

Seeds large, 1–1.5 mm. long; plant usually subglabrous; leaves usually subcoriaceous, subentire. So. Ecuador to Bolivia ..... 1a. *Oe. multicaulis* var. *typica*

Seeds smaller, 0.5–0.9 mm. long; plant usually villous in upper parts; leaves thick-herbaceous, somewhat denticulate. Central Ecuador to Mexico.

1b. *Oe. multicaulis* var. *tarquensis*

1a. **Oenothera multicaulis** R. & P. var. **typica** n. nom.

Oe. multicaulis R. & P., l.c. *Xylopleurum multicaule* Loesener in Fedde, Repert. **12**: 237. 1913. *Oe. multicaulis* var. *petiolaris* Ball, Jour. Linn. Soc. **22**: 38. 1885. *Oe. Barbeyana* Lévl., Monde des Plantes **8**: pl. 109. 1898. *Hartmannia boliviana* Rusby, Descr. S. Am. Pls., 72. 1920.

Stems 5–10(15) cm. long, usually subglabrous; leaves somewhat coriaceous, the cauline ones 5–15 mm. long, usually entire; capsules narrow, barely 3–4 mm. thick, including wings; seeds 1–1.5 mm. long.

Type locality, "in Peruviae Andium subalpinis Tarmae et Cantae Provinciarum." Growing at high altitudes in Peru and Bolivia. Representative of material seen, PERU: Nueva Espana, Herb. *Pavon* (Boiss.); Cuzco, *Herrera in 1923* (Ber, US); Chicla, *Ball in 1882* (K); Rio Blanco, *Macbride & Featherstone 746* (G, K); Chacapoyas, *Matthews* (Ber, Boiss, K). BOLIVIA: Sorata, *Mandon 632* (BM, Del, K, G, NY); Songo, *Bang 914*, type coll. *Barbeyana* (Ber, BM, Boiss, E, G, M, NY, US); Lake Titicaca, type coll. *H. boliviana*, *Buchtien 2922* (US); Quaqui, *Hill 161* (K); La Paz, *Hauthal 181* (Ber).

From Cuenca, Ecuador, *Rose, Pachano & Rose 22942* (US) and from Zaragura, Loja, Ecuador, *Rose, Pachano & Rose 23163* (G, US) comes material that is quite intermediate between *typica* and *tarquensis* in fruit, pubescence, etc.

1b. OENOTHERA MULTICAULIS var. TARQUENSIS (HBK) Munz & Johnston, Contr. Gray Herb., n. ser. **75**: 18. 1925

Oe. tarquensis HBK, Nov. Gen. et Sp. **6**: 91. 1823. *Hartmannia tarquensis* Spach, Nouv. Ann. Mus. Paris **4**: 363. 1835. *Oe. ascendens* Willd., in Spreng., Syst. **2**: 230. 1825. *Oe. aurantia* Willd., l.c., in synonymy. *Oe. cuprea* Schlecht., Linnaea **12**: 269. 1838. *Hartmannia cuprea* Rose, Contr. U. S. Nat. Herb. **8**: 328. 1905. *Oe. epilobiifolia* HBK., Nov. Gen. et Sp. **6**: 92. 1823. *Hartmannia epilobifolia* Spach, Nouv. Ann. Mus. Paris **4**: 364. 1835.

Stems 10–30 cm. long, villous; cauline leaves more or less villous, especially the upper ones, herbaceous, commonly 2–3 cm. long, usually denticulate; capsules 4–5 mm. thick, villous; seeds small, 0.5–0.9 mm. long.

Type locality, "prope urbem Quito, in convalli Tarquensi." Representative of material seen, without locality, Hort. Berol in 1846, *Oe. cuprea*, ex herb. Kunth (Ber); Nova Granata, *Oe. epilobiifolia*, Herb. Bonpland (Par, photo at P); *Oe. ascendens* and *Oe. aurantia*, Humboldt, Willdenow Herb. (Ber). ECUADOR: Valle de Tarqui, herb. Bonpland (Par); Llalla *Spruce 5059* (Boiss, BM, Del, G, K, E, Par); Quito, *J. N. & G. Rose 23533* (G, US), *Jameson 191* (BM, Del, K); sive Andium Quitensium, *Jameson 355* (Del, E, Par); Pichincha, *Couthouy in 1855* (G, NY). COLOMBIA: Bogotá, *Pennell 2330* (G, US); above Suratá, dept. Santander, *Killip & Smith 16738* (G, NY, US); Parama de Maenchies, *Moritz 1253* (Ber). VENEZUELA: Caracas, *Linden 450* (Del, K, Par); Paramo de Santo Domingo, Mérida, *Jahn 593* (US). GUATEMALA: Volcan de Agua, Zacatepequez, *Donnell Smith 2149* (Ber, G, K, US); between Nahualá & Quezaltenango, *Seler 2947* (Ber). COSTA RICA: Westhang des Irazú, *Brade 2019* (Ber), *Dodge 3427* (G); Paturage au Litio Binis, *Pittier 43* (K). MEXICO: Sierra de San Felipe, Oaxaca, *Pringle 4702* (Ber, BM, Del, E, G, K, US); Orizaba, *Galeotti 3027* (Del, K); Sierra de Pachuca, Hidalgo, *Rose, Painter and Rose 8863* (US).

2. **Oenothera deserticola** (Loesener) Munz, n. comb.

Xylopleurum deserticola Loesener, in Fedde Rep. **12**: 238. 1913. *Hartmannia montana* Rose, Contr. U. S. Nat. Herb. **8**: 329. 1905. Not *Oenothera montana* Nutt. 1840.

Perennial herb from woody caudex, with 1 to few pilose, decumbent, somewhat purplish stems 1–4 dm. long; leaves subentire, subglabrous above, or with scattering soft hairs on both surfaces, but especially along the margins and veins; basal leaves oblanceolate, 5–10 cm. long, obtuse, narrowed into short petiole; cauline lance-ovate, somewhat oblong, obtuse to acute, mostly 3–7 cm. long, nearly sessile; flowers solitary in upper axils, diurnal?; calyx-tube pilose without, pubescent within near the base, 5–8 mm. long; calyx-lobes pilose, usually coherent in anthesis, 1.5–2 cm. long, with free tips in the bud 1–2 mm. long; petals white, changing to purple, 15–18 mm. long; stamens subequal, ca. half as long as the petals; anthers 4–5 mm. long, on filiform filaments 6–7 mm. long; style slightly shorter than stamens, pubescent at very base; stigma-lobes linear, ca. 4 mm. long; capsule scarcely arcuate, obovoid, pilose, 1.5–2 cm. long, 6–8 mm. thick, with wings 1.5–3 mm. wide, and passing gradually into a stout pedicel 2–6 cm. long; seeds brown, asymmetrically oblong-obovoid, 1.2 mm. long.

Type locality, Mexico, in the Federal District. Material seen, VERA CRUZ: Mt. Orizaba, *Rose & Hay 5672* (US). MEXICO: Cima, *Rose & Painter 7170*, type coll. *montana* (G, US); La Cima de Ajusco, *Pringle 10293* (Ber, BM, Boiss, Del, E, G, K, US); Cima Station, *Pringle 13689* (G); without locality, *Uhde 1222* (Ber); El Desierto, valley of Mexico, collected

by *Hopfner*, *Seler* distribution no. *5614* (Ber); San Rafael, Tlalmancalo, *Seler 5319* (Ber); El Desierto, *Seler 5280*, type specimen (Ber).

3. **Oenothera Purpusii** Munz, n. sp.

Perennial, even suffrutescent, more or less caespitose, the stems 5–10 (15) cm. long, decumbent or ascending, canescent with a short subappressed pubescence; radical leaves 1–2.5 cm. long, oblanceolate to obovate, short-petioled, subentire or remotely denticulate, obtuse, subglabrous or somewhat pubescent on margins and veins; cauline leaves oblanceolate, short-petioled or subsessile, 1–2 cm. long, sinuate-denticulate, obtuse to acute, more or less gray-pubescent; flowers few, solitary in upper axils; calyx-tube and ovary pilose, the former 6–8 mm. long, minutely pubescent within near the base; calyx-lobes appressed-canescent, ca. 1 cm. long, without free tips in bud, more or less coherent in anthesis; petals apparently white, changing to purple, broadly obovate, 12–14 mm. long; stamens half to two-thirds the petals, subequal; anthers 2.5–3 mm. long; filaments filiform, 5–7 mm. long; style equaling or exceeding stamens, pubescent at very base; stigma-lobes linear, 1.5–2 mm. long; capsule narrowly obovoid, pubescent, 1–1.5 cm. long, 4–6 mm. thick, on a pedicel 5–10 mm. long, 8-angled, but scarcely winged; seeds brown, narrow-obovoid, 1–1.2 mm. long. (Perennis, caespitosa; caulibus 5–10(15) cm. longis, subdecumbentibus, canescentibus; foliis 1–2.5 cm. longis, oblanceolatis aut obovatis, subintegris aut denticulatis, obtusis, subglabratis; floribus paucis, solitariis; hypanthio piloso, 6–8 mm. longo; sepalis canescentibus, 1 cm. longis; petalis albis, late obovatis 12–14 mm. longis; staminibus subequalibus, 7–10 mm. longis; lobis stigmatis linearibus, 1.5–2 mm. longis; capsulo anguste obovoideo, pubescente, 1–1.5 cm. longo, 8-angulato, vix alato; seminibus brunneis, anguste obovoideis, 1–1.2 mm. longis.)

Type, from open woods, Ixtaccihuatl, Mexico, *C. A. Purpus 1839*, Pomona College Herbarium Number 32827; isotypes at Gray and US. Other material, Salazar, Mexico, *Rose and Painter 7027* (US); Mt. Popacatapetl, *E. W. Nelson 10* (US). MICHOACAN: Toluca, *Rose & Painter 7873* (US), *Andrieux 385* (G, K). HIDALGO: Sierra de Pachuca, *Rose & Hay 5611* (US).

Characterized among the decumbent species of Hartmannia in Mexico by the appressed canescence, the lack of wings on the fruits, and by the sinuate-denticulate leaves.

4. **Oenothera Kunthiana** (Spach) Munz, n. comb.

Hartmannia Kunthiana Spach, Nouv. Ann. Mus. Paris 4: 363. 1835. *Oenothera pinnatifida* HBK., Nov. Gen. et Sp. 6: 91. 1823, not Nutt. 1818. *Oe. Humboldtii* Engelm., Amer. Journ. Sci. II, 34: 334. 1862. *Oe. pinnatifida* Hort. nec. HBK., Spach, l.c. *Hartmannia parviflora* Spach, l.c. *Oe. fissifolia* Steud., Nom. ed. II, 2: 206. 1841. *Oe. micrantha* Walp., Rep. 2: 84. 1843. *Oe. Walpersii* Donn. Smith, Enum. Pl. Guat. 4: 60. 1895. *Oe. tetraptera* var. *Childsii* Bailey, Cyclop. Amer. Hort., 1121. 1901. *Oe. tetraptera* forma *depauperata* Lévl., Mon. Onoth., 122. 1902. *Oe. t.* f. *roseoidea* Lévl., l.c. *Oe. tetraptera* var. *rosea* Lévl., 449. 1913, nom. nud.

Perennial herb, usually with several to many stems from summit of a taproot, the stems slender, decumbent or ascending, 0.5–3(6) dm. long, crisped pubescent with some long spreading hairs, and usually appressed-canescent in upper parts; leaves of basal rosette oblanceolate, 3–10 cm. long, 1–2 cm. wide, usually sinuate-pinnatifid into several ovate to lanceolate, acute, lateral lobes 1–8 mm. long and with a much larger rhomboid, ovate terminal lobe; cauline leaves reduced, mostly 1–3 cm. long, lobed as above, or sinuate-dentate, or subentire; uppermost becoming leafy, lanceolate bracts ca. 1 cm. long; flowers few per stem, solitary in upper axils, sessile or short-pedicelled; calyx-tube 1–2 cm. long, strigulose, sometimes also with spreading hairs, puberulent within at very base; calyx-lobes lance-linear, ca. 1 cm. long, usually coherent at least in pairs in anthesis, without free tips; petals whitish or pink, 10–15(20) mm. long, obovate; stamens subequal, ca. two-thirds as long as the corolla; anthers 3–4 mm. long, filaments 5–10 mm. long; style as long as or exceeding stamens, puberulent at very base; stigma-lobes linear, 3–5 mm. long; capsule obovoid, the body proper ca. 1 cm. long, 4–5 mm. thick, 4-ribbed and 4-winged, the wings ca. 1 mm. wide, more or less hirsute; capsule passing below into a slender pedicel, 0.5–3 or more cm. long; seeds brown, asymmetrically obovoid, ca. 1 mm. long.

Type locality, Actopan, Mexico. Ranging from Texas to central Mexico. Representative material may be cited as follows: TEXAS: San Antonio, *Clemens 678* (P); Glenn Springs, Brewster Co., *Cory 2116* (P); Stewarts, Brewster Co., *Cory 2121* (P); Laredo, *Reverchon 3553* (G, US); Browns, Terrell Co., *Cory V7* (P); Ft. Clark, Kinney Co., *Mearns 1277* (US); Bofecillos Mts., *Havard 190* (G); Eagle Pass, *Havard 3* (G). SONORA: Alamos, *Rose, Standley & Russell 13032* (G, US); Hermosillo, *Rose et al. 12406* (US); Mazatlan, *Rose et al. 14165* (US). CHIHUAHUA: Bachimba Canyon, *Pringle 161* (Ber, Boiss, BM, Del, K, Par, US). COAHUILA: Saltillo, *Palmer 568*, in 1898 (US); Sierra de la Paila, *Endlich 835* (Ber). NUEVO LEON: Monterey, *Palmer 347*, in 1880 (G, K, US). TAMAULIPAS: Tampico, *Palmer 563*, in 1910 (BM, Del, K, G, US); Victoria, *Palmer 114*, in 1907 (G, K, US). SAN LUIS POTOSI: San Luis Potosi, *Parry & Palmer 250*, in 1878 (BM, E, G, K, Par, US); Taupamolon, *Seler 230* (Ber). ZACATECAS: Ojo Caliente, *Jones in 1892* (P). DURANGO: Durango, *Palmer 27*, in 1896 (BM, Boiss, G, K, US). VERA CRUZ: Jalapa, *Rose & Hay 6364* (US). MORELOS: Cuernavaca, *Schaffner 533* (BM). OAXACA: Oaxaca, *Conzatti 87* (G). HIDALGO: Actopan, Bonpland Herbarium (Par) and another sheet (Ber), type collection. Naturalized to some extent in South Africa, NATAL: Meritzburg, *Rudatis 1605* (BM); Basutoland, *Dieterlen 327* (BM).

Intergrading freely with *Oe. tetraptera*, but its smaller, pinker flowers, more prostrate habit, longer calyx-tube, smaller fruit, finer more appressed pubescence—all these argue for recognition. As representative of intergrades with *Oe. tetraptera* may be cited *Palmer 140* from San Dieguito, San Luis Potosi (G, US); *Seler 2144* from Chiapas (Ber); *Heyde & Lux 2922*, San Miguel Uspantan, Quiché, Guatemala (Ber, BM, G, K, US). These are quite intermediate in having rather large flowers and fruits and with considerable pilosity.

5. OENOTHERA TETRAPTERA Cav., Ic. 3: 40, pl. 279. 1794

Xylopleurum tetrapterum Raim., in Engler & Prantl, Die nat. Pflanzenfam. III, 7: 214. 1893. *Hartmannia tetraptera* Small, Bull. Torrey Bot. Club 23: 181. 1896. *Oe. tetraptera* f. *chenopodifolia* Lévl., Mon., 122. 1902. *Oe. tetraptera* var. *immutabilis* Lévl., Mon., 122. 1902. *Hartmannia macrantha* Spach, Nouv. Ann. Mus. Paris 4: 363. 1835. *Oe. latiflora* Ser. in DC., Prodr. 3: 50. 1828. *Hartmannia latiflora* Rose, Contr. U. S. Nat. Herb. 8: 329. 1905. *Oe. capensis* Hort. & *Oe. dubia* Hort. ex Steud., Nom. ed. 2, 2: 206. 1841.

Perennial herb, sometimes suffrutescent at base, decumbent to ascending, with few simple or branched stems 1.5–5 dm. long, more or less strigulose and also with long spreading hairs; basal leaves oblanceolate or broader, 3–10 cm. long, 1–3 cm. wide, sinuate-pinnatifid into several ovate-oblong lateral lobes and larger terminal one, or even quite entire, with well developed petioles, subglabrous to villous; cauline leaves reduced, 2–5 cm. long, commonly lance-ovate and irregularly sinuate-pinnatifid, sometimes merely sinuate-dentate or even subentire; uppermost lanceolate, acuminate, 1–2 cm. long, with flowers in axils; flowers vespertine; calyx-tube commonly hirsute as well as strigose, ca. 1 cm. long, puberulent within at base; calyx-lobes commonly reddish, 2–3.5 cm. long, coherent in anthesis, at least in pairs, with minute free tips in bud; petals pink or usually whitish, darker with age, 2–3.5 cm. long, broadly obovate; stamens subequal, one-half to two-thirds the length of the petals; filaments 1–1.5 cm. long; capsule proper obovoid, 1–1.5 cm. long, 6–8 mm. thick, hirsute, especially on midveins and wings, the latter 2–3 mm. wide; capsule narrowed into ribbed hollow pedicel 5–25 mm. long; seed light brown, obovoid, ca. 1.3 mm. long.

Type locality, "Sotoluca, Nova Hispaniaë." Ranging from Mexico to Colombia and Venezuela, and widely escaped in many other parts of the world. Representative of material seen, MEXICO, without locality, drawing of *Oe. latiflora* Seringe (Del, G, P). COAHUILA: Sierra Madre, so. of Saltillo, *Palmer 340*, in 1880 (G, K, US). NUEVO LEON: Monterey, *Palmer 335*, in 1880, in part (G, K, US). HIDALGO: Sierra de Pachuca, *Rose & Hay 5638* (US); Tula, *Rose, Painter and Rose 8322* (US). MEXICO: Chapultepec, *Aschenborn 511* (Ber); Valley of Mexico, *Bourgeau 300* (Ber, Boiss, Del, G, K, Par, US); Ixtaccihuatl, *Purpus 210* (US). PUEBLA: near Puebla, *Arsène 9841* (US). VERA CRUZ: Jalapa, *Linden 623* (K). OAXACA: Los Narranjas, *Purpus 4235* (BM, G, US). CHIAPAS: Chiapas, *Ghiesbreght 680* (BM, Boiss, G). GUATEMALA: Llano, *Bernouilli 350* (Ber, K); Aguas Calientes, *Deam 6114* (US). COSTA RICA: Cartago, *Stork 306* (US); San José, *Buckley 1016* (US). COLOMBIA: Suratá, Santander, *Killip & Smith 16485* (G, NY, US). VENEZUELA: Tovar, *Fendler 451*, type coll. f. *chenopodifolia* (Boiss, Del, G, K); Sierra Nevada de Mérida, *de Bellard 233* (US). I have seen naturalized plants from Jamaica, India, East Africa, South Africa. I have seen also the following sheets of interest: *Oe. tetraptera*, misit Cavanilles, without flowers, but with fruits 8 mm. thick (Del); *Oe. Reichenbachii*, *Oe. capensis* Reichb., Herb. K. Sprengel (Ber); *Oe. dubia* Hort., Hort. bot. Berol. (Ber); *Oe. tetraptera*, C. G. Ortega, Lady Bute (BM).

6. OENOTHERA ROSEA Aiton, Hort. Kew. ed. 1, 2: 3. 1789

Hartmannia rosea G. Don in Sweet, Hort. Brit. ed. 3, 236. 1839. *Xylopleurum roseum* Raim., in Engler & Prantl, Die nat. Pflanzenfam. III, 7: 214. 1893. *Oenothera rubra* Cav., Icones 4: 68, t. 400. 1797. *Oe. purpurea* Lam., Encycl. 4: 554. 1797. *Oe. virgata* R. & P., Fl. Peruv. 3: 79, t. 315. 1802. *Hartmannia virgata* Spach, Nouv. Ann. Mus. Paris 4: 363. 1835. *H. gauroides* Spach, l.c. *H. affinis* Spach, Ann. Sci. Nat. ser. 2, 4: 167. 1835. *Oenothera psycrophila* Ball, Jour. Linn. Soc. 22: 38. 1885. *Oe. rosea* var. *parvifolia* Coulter, Contr. U. S. Nat. Herb. 2: 116. 1891. *Hartmannia rosea* var. *parvifolia* Small, Bull. Torrey Bot. Club 23: 181. 1896. *Oe. lyrata* Lévl., Mon. Onoth., 11, 1902, in syn. *Godetia Heucki* Phil., Anal. Univ. Chil. 84: 743. 1893. *Gaura epilobia* Moc. & Sesse, ex Ser. in DC., Prodr. 3: 45. 1828.

Perennial, blooming the first year, with several erect or ascending, slender, simple or branched stems, from a somewhat woody caudex and 1–5 dm. tall, or shrubby and becoming a meter or so tall, more or less strigulose throughout, sometimes with spreading hair on the lower stems; leaves usually not crowded, the basal ones oblanceolate to narrowly obovate, subentire to coarsely sinuate-dentate or -pinnatifid, 2–5 cm. long, obtuse, narrowed into slender petioles 1–2 cm. long; cauline leaves gradually reduced up the stem, mostly oblong-ovate, subentire to sinuate-denticulate or even somewhat pinnatifid at base of blade, 1.5–3 cm. long, obtuse to acute; uppermost reduced to greenish lance-linear bracts, in axils of which are the flowers in slender racemes; calyx-tube 4–8 mm. long, slender, strigulose-canescent without, somewhat puberulent within; calyx-lobes 5–8 mm. long, with free tips ca. 1 mm. long and commonly coherent in anthesis; petals rose to red-violet, 5–10 mm. long, broadly obovate; stamens subequal; almost as long as petals; anthers 2.5–4 mm. long, on slender filaments 4–6 mm. long; style scarcely equaling stamens, puberulent at very base; stigma-lobes linear, ca. 2 mm. long; capsule proper obovoid, 8–10 mm. long, 3–4 mm. thick, strigulose, the 4 angles somewhat winged, scarcely 1 mm. wide, the mid nerve of each valve strongly developed, base of capsule passing into a hollow and ribbed pedicel 5–20 mm. long; seeds oblong-obovoid, asymmetrical, brown, ca. 0.6 mm. long.

Type locality, "Nat. of Peru." Apparently native from the southwestern United States to Peru and Bolivia; widely introduced into many other parts of the world. Representative of material seen, *Oenothera rosea* sp.n., *Kew 1786*, herb. of the late Bishop Goodenough (K); *Oe. rosea*, *Hort. Paris, 1785* (Par); *Hort. Kew*, 1784 (BM); Hort. Paris, e Peru (Dombey), *Mrs. L'Heritier, 1784* (BM); *Oe. purpurea*, Dict. n. 10, *Lamarck Herb.* (Par). TEXAS: Limpia Canyon, W. Tex., *Nealley 652*, type of var. *parvifolia* (US). NEW MEXICO: without locality, *Wright 1071*, in 1851 (BM, G, K, Par, US). ARIZONA: Ft. Huachuca, *Lemmon 2704* (BM, Del, G, US); Sacaton, *Harrison 1778* (US); Ft. Lowell, *Thornber 457* (US). CHIHUAHUA: Canyon of Rio San Miguel, *Hartman 652* (G, K, US); Chihuahua, *Palmer 6*, in 1908 (Ber, G, K, US). COAHUILA: Saltillo, *Gregg 254* (Ber, G); Sabinas, *Endlich*

796 (Ber). NUEVO LEON: Monterey, *Arsène 6181* (US). DURANGO: Santiago Papasquiaro, *Palmer 46*, in 1896 (Ber, BM, G, K, US); San Ramón, *Palmer 130*, in 1906 (G, K, US). SINALOA: Culiacan, *Rose, Standley & Russell 14041* (US). SONORA: Santa Cruz, *Mearns 2631* (US). GUANAJUATO: Guanajuato, *Dugès 306* (G). JALISCO: Between Santa Gertrudis and Santa Teresa, *Rose 2082* (US). ZACATECAS: San Miguel, *Jones 234* (P, US). SAN LUIS POTOSI: San Luis Potosi, *Parry & Palmer 251* (E, G, K in part, US); Morales, *Schaffner 170* (G, K). TAMAULIPAS: Victoria, *Palmer 171* (K, US). PUEBLA: Toluca, *Andrieux 386* (G, K). VERA CRUZ: Orizaba, *Seaton 85* (G, US). MEXICO: Mexico, Bourgeau 39 (Ber, Del, G, K, Par, US). OAXACA: La Parada, *Nelson 1030* (US); Oaxaca, *Deam 13* (G, US). GUATEMALA: Coban, Alta Verapaz, *Türckheim 11624* (BM, Del, E, G, US); San Miguel Uspantán, *J. Donnell Smith 2876* (Ber, E, G, US). COSTA RICA: Cartago, *Cooper 5769* (Ber, Boiss, G, K, US); San José, *Tonduz 441* (Boiss, US). ECUADOR: Andes of Quito, *Jameson 791* (BM, Del, K); in Andibus, *Spruce 5963* (BM, Del, E, G, K, Par). BRAZIL: Sorocaba, S. Paulo, *Wacket in 1902* (Ber); Rio Janeiro, *Glaziou 8342* (Ber, K, Par, US); very probably introduced. PERU: Chicla, *Ball in 1882*, type of *psycrophila* (K, photo at G, P); Huanuco, *Ruiz*, as *virgata* (Ber); Nouvelle Espagne, as virgata, *Pavon* (Del); San Sebastian, Cuzco, *Pennell 13609* (G, NY, US). BOLIVIA: Cotaña, *Buchtien 148* (Ber, Del, E, G); Soratá, *Rusby 1814* (G, K, US); Capi, *Bang 779* (Ber, BM, Boiss, E, G, K, M, US). CHILE: (where it must be introduced), Parque Cousino, Santiago, *Gigone in 1886* (G); San Felipe, *Claude-Joseph 1698* (US); Limache, Santiago, *Garaventa 943* (G); Nuñoa, *Philippi*, as Godetia Heucki (BM). I have seen material of *Oenothera rosea* from Canary Islands, Azores, France, India, Rhodesia, Transvaal, etc.

7. OENOTHERA SPECIOSA Nutt., Journ. Philad. Acad. 2: 119. 1821

Perennial, from a running rootstock, sometimes suffrutescent, with one to several stems 1–5 dm. long, erect or ascending or almost prostrate, simple or branched, usually strigose, sometimes with spreading hairs, leafy; leaves glabrous to sparsely strigulose, the basal ones oblanceolate to obovate, 1–several cm. long, petioled, usually sinuate-pinnatifid into lanceolate or ovate lateral lobes and larger terminal one; cauline leaves oblong-lanceolate to -ovate, 3–8 cm. long, short-petioled, the lower ones usually more or less deeply pinnatifid, upper ones sinuate-dentate or subentire and often narrower, few uppermost reduced to lance-linear bracts ca. 1 cm. long; flowers few to several, solitary in uppermost axils, vespertine, with nodding buds; calyx-tube strigose without, 1–2 cm. long, pubescent within at base; calyx-lobes strigose, lance-linear, long-acuminate, 1.5–3 cm. long, coherent in anthesis, with free tips 0.5–4 mm. long; petals white to pink, obcordate, 2–4 cm. long; stamens subequal, half as long as petals; stamens 5–10 mm. long; filaments 10–17 mm. long, filiform; style exceeding stamens, pubescent at very base; stigma-lobes linear, 3–6 mm. long; entire capsule 10–15 mm. long, the terminal fertile part 2–5 mm. thick, appearing 8-ribbed, with strong veins and wings less than 1 mm. wide, attenuate into cylindrical

basal part 1.5–2 mm. thick, sessile; seeds brown, asymmetrically obovoid, ca. 1 mm. long.

KEY TO VARIETIES

Stems erect or nearly so; petals 2.5–4 cm. long; capsule 3–5 mm. thick (including the wings). 7*a*. *Oe. speciosa* var. *typica*

Stems prostrate; petals 2–2.5 cm. long; capsule 2–3 mm. thick (including the wings). 7*b*. *Oe. speciosa* var. *Berlandieri*

7*a*. **Oenothera speciosa** Nutt. var. **typica** Munz, n. nom.

Oenothera speciosa Nutt., l.c. *Xylopleurum speciosum* Raimann, in Engler & Prantl, Die nat. Pflanzenfam. III, 7: 214. 1893. *Hartmannia speciosa* Small, Bull. Torrey Bot. Club 23: 181. 1896. *Oe. speciosa* var. *serrulatiformis* Lévl., Mon. Onoth., 119, 1902, in syn. *Oe. speciosa* f. *subintegrifolia* Lévl., Mon. Onoth., 118, 1902. *Xylopleurum Nuttallii* Spach, Nouv. Ann. Mus. Paris 4: 371. 1835. *X. hirsutum* Spach, l.c., 370. *Oenothera hirsuta* Steud., Nom. ed. II, 2: 206. 1841. *X. Drummondii* Spach, l.c., 371. *X. obtusifolium* Spach, l.c., 372. *Oenothera obtusifolia* D. Dietr., Syn. Pl. 2: 1289. 1840. *Oe. Spachii* D. Dietr., l.c. *Oe. Webbiana* Steud., l.c., 208. *Oe. Shimeki* Lévl., Mon. Onoth., 119. 1902.

Stems rather coarse, 2–3 mm. thick, erect or ascending; cauline leaves 3–8 cm. long, oblong; flowers white to pink; petals 2.5–4 cm. long; capsules stout, 3–5 mm. thick.

Type locality, "On the plains of the Red River"; type collected by Nuttall. With two apparent forms: one low, 1–3 dm. tall, and with several stems from the base, these ascending; the other form taller, 3–6 dm. high, and with one to few erect stems. Since these two growth forms intergrade so completely in any given locality, I have been unable to separate them for varietal recognition. Var. *typica* ranges from Kansas and Missouri to Texas and northeastern Mexico; it has become established also in other places. Representative of material seen, KANSAS: Chatauqua Co., *Hitchcock 160a* (G, Par); Riley Co., *Norton 160* (G, US); Wichita, *Poole 259* (G). OKLAHOMA: Hinton, Caddo Co., *Stevens 930* (G, K); Mannsvile, Johnston Co., Coll. Griffith, distributed by *Stevens* as *3490* (G); Sapulpa, *Bush 167* (M); Oklahoma City, *Shimek in 1892*, type of Shimeki (M). TEXAS: Bejar, Mars, 1828, *Berlandier 1447* (Boiss, Del) labeled X. hirsutum by Spach; San Antonio de Bejar, Martio 1828, *Berlandier 187* (G); Rio Brazos, *Drummond 9* (K), *33* (K), *78* (G), without locality, probably from San Felipe, *Drummond 79* (Del, E, G, K); New Braunfels, *Lindheimer 409* (G, K); Comanche Spring, *Lindheimer 811* (Ber, BM, G, K, Par); Dallas, Reverchon coll., *Curtiss dist. no. 913* (Ber, Boiss, BM, G, K, US); San Antonio, *Heller 1703* (Boiss, G, K, US); Houston, *Lindheimer 55* (Ber, Boiss, G, Par). ARKANSAS: Red River, *Nuttall* (K), Arkansas Terr., *Nuttall* (BM). COAHUILA: Rio Grande Valley near Diaz, *Pringle 8305* (Ber, Boiss,

BM, Del, G, K, P, Par, US). CHIHUAHUA: Chihuahua, *Palmer 186*, in 1908 (K, US). DURANGO: Durango, *Palmer 100*, in 1896 (Boiss, G, K, US). SAN LUIS POTOSI: Rio Verde, *Palmer 42* (K, US). I have seen material also from California, Lower California, Illinois, Missouri, Louisiana, Georgia, Alabama, North Carolina, etc., where it is apparently introduced.

Other tendencies to variation beside the two in habit above mentioned are to be found in the size and color of the flowers, in the shape of the leaves (from narrow to oblong, entire to pinnatifid), and in the pubescence (from appressed to spreading). But on the whole the large white flowers, drooping buds, ascending to erect stems, and stout cylindrical base of the fruit are amply characteristic.

There is more or less intergradation with var. *Berlandieri* as evidenced by *Reverchon 3555* from Laredo, Texas (US) with slender stems and petals 3.5 cm. long but pink; by *Nealley in 1889* from Santa Maria, Texas (US), with habit of *typica*, but petals rose and 20 mm. long; by *Rose 4171*, from Houston, Texas (US), with petals 24 mm. long, but habit of *typica*.

7*b*. **Oenothera speciosa** Nutt. var. **Berlandieri** (Spach) Munz, n. comb.

Xylopleurum Berlandieri Spach, Nouv. Ann. Mus. Paris 4: 370. 1835. *Oenothera Berlandieri* Walp., Rep. 2: 85. 1843. *Oe. Delessertiana* Steud., Nom., ed. II, 2: 206. 1841. *Hartmannia Berlandieri* Rose, Contr. U. S. Nat. Herb. 8: 328. 1905. *H. Reverchoni* Rose, l.c. 329. *Oenothera speciosa* f. *variegata* Lévl., Mon. Onoth., 118. 1902. *Oe. speciosioides* Lévl., l.c., 113. *Oe. tetrapteroides* Lévl., l.c.

Stems very slender, 1–1.5 mm. thick, prostrate or decumbent, usually 1–1.5 dm. long, sometimes pilose as well as appressed-pubescent; leaves usually not more than 3–4 cm. long; flowers rose-color, the petals 2–2.5 cm. long; capsules slender, 2–3 mm. thick.

Type locality, "Crescit in Regno mexicano," probably Matamoros. Not too clearly marked as a variety and intergrading freely with var. *typica*, but in its smaller redder flowers, more slender stems, and more prostrate habit, the plant of the immediate coastal region of Texas, from Corpus Christi and Galveston to adjacent Mexico, seems worth some recognition. Representative of material seen, TEXAS: Galveston, *Benke 4604* (G); Rio Hondo, Cameron Co., *Chandler 7013* (Ber, G, US); Corpus Christi, *Heller 1406* (Boiss, BM, Del, G, K, P, Par, US); McAllen, Hidalgo Co., *Parks 2932* (P); Laredo, *Berlandier 224* = *1484* (G). TAMAULIPAS: near Matamoros, *Berlandier 2289* = *869* (Del, G, K), 2326 = 896 (BM, Boiss, Del, G, K), one of the sheets of the latter collection at the Delessert Herbarium bears the name *H. Berlandieri* in Spach's writing. Cultivated, at Missouri Botanical Garden in 1894, *x speciosoides* (M).

GAUROPSIS[1]

Gauropsis (Torr. & Frem.) Munz, n. subgenus

Gauropsis, as subsect., Torr. & Frem., Rep., 315. 1845; as genus, Cockerell, Bot. Gaz. 30: 351. 1900. *Gaurella*, as genus, Small, Bull. Torrey Bot. Club 23: 183. 1896.

Low bushy perennial herbs, with branching stems and small entire or dissected leaves. Flowers white or yellow to rose, often with reddish stripes or spots, axillary. Calyx-tube cylindric, as long as or exceeding the calyx-lobes. Stamens subequal. Stigma 4-lobed. Capsule ovoid-pyramidal or oblong-ovoid, sessile, attenuate at each end, with its angles keeled or winged, the valves strongly ribbed; seeds obovoid, angled, in several rows in each cell.

The species referred to this subgenus in this paper have been variously placed, sometimes in the subgenus Megapterium (from which they differ in having the seeds in more than 2 rows in each cell and in having the seeds angled and not corky) and sometimes in the subgenus Hartmannia (from which they can be separated by having the capsule ovoid or oblong-ovoid and not clavate). In fact, the three species here treated are not closely related to any of the other subgenera, but seem to constitute a group of their own.

Key to Species

Leaves pinnatifid, 1–5 cm. long; petals 1.5–3 cm. long; capsule 10–15 mm. long.
- Stigma-lobes 2–3 mm. long; leaves with 3–4 pairs of lobes 2–3 mm. long; capsules angled but not winged ..... 3. *Oe. Havardi*
- Stigma-lobes 5–8 mm. long; leaves with several pairs of lobes 5–8 mm. long; capsules with wings 2–3 mm. wide ..... 2. *Oe. dissecta*

Leaves denticulate, 0.5–1.5 cm. long; petals 6–10 mm. long; capsule 6–8 mm. long. 1. *Oe. canescens*

Treatment of Species

1. Oenothera canescens Torr. & Frem., Rep., 315. 1845

Megapterium canescens Britton, Mem. Torrey Bot. Club 5: 235. 1894. *Gaurella canescens* A. Nels., in New Man. Bot. Centr. Rocky Mts., 341. 1909, and Bot. Gaz. 47: 347. 1909. *Gauropsis canescens* Cockerell, Bot. Gaz. 30: 351. 1901. *Oenothera guttulata* Geyer ex Hook, London Journ. Bot. 6: 222. 1847. *Gaurella guttulata* Small, Bull. Torrey Bot. Club 23: 183. 1896. *Gauropsis guttulata* Cockerell, l.c.

Bushy perennial herb, diffusely branched from near base, 1–2 dm. high, the branches decumbent or ascending, appressed-canescent throughout; leaves lanceolate or linear-lanceolate, almost sessile, repand-denticulate to almost entire, 5–15 mm. long, with small fascicles in the axils; flowers

[1] The name Gauropsis was used by Presl (Epim. Bot., 219. 1849) as a genus name for a new species *lancifolia*. In Oct., 1931, I had the privilege of examining Presl's type at the German University in Prague. I found it to be a perfectly clear specimen of *Clarkia elegans* Dougl. and can definitely throw it into synonymy.

sessile; calyx-tube 5–8 mm. long, strigulose; calyx-lobes lanceolate, coherent in anthesis, 8–9 mm. long, canescent, without free tips; petals white to pink, with red spots or stripes, obovate, 8–11 m. long; stamens subequal, two-thirds the petals; anthers 4–5 mm. long; style equaling the stamens or even the petals; stigma-lobes 2 mm. long; capsule ovoid-pyramidal, sessile, sharply 4-angled, 7–8 mm. long, canescent, somewhat beaked; seeds obovoid, 1 mm. long, somewhat slipper-shaped.

Type locality, "upper waters of the Platte." Found in dried up ponds, on the western plains from Wyoming and Colorado to Kansas, New Mexico and western Texas. Representative of material seen, WYOMING: Alden, *Vasey* (G). COLORADO: between Keota & Briggsdale, Weld Co., *Osterhout 6034* (P). KANSAS: buffalo wallows, Finney Co., *Hitchcock 162* (G, US); Syracuse, *Thompson 143* (US); plains of Upper Platte, *Geyer 178* (Del, K); upper waters of the Platte, *Fremont in 1843–4*, type coll. (G). NEW MEXICO: Rock Creek, *Wislizenus* (G); Sierra Grande, *Howell 216* (US); Sand Creek of the Cimarron, *Fendler 227* (G); Enchanted Spring, Santa Fe Trail, *Newberry* on Macomb Exped. (G). TEXAS: Amarillo, *Reverchon 3840* (G, P, US); Tahoka, *Tharp 4442* (US).

2. OENOTHERA DISSECTA Gray, ex Wats., Proc. Amer. Acad. **17**: 357. 1882

Megapterium dissectum Small, Bull. Torrey Bot. Club **23**: 184. 1896.
Hartmannia dissecta Rose, Contr. U. S. Nat. Herb. **8**: 328. 1905.

Perennial from underground rootstocks, with stems up to 2 dm. long, slender, branched, quite leafy, more or less strigulose throughout; leaves lanceolate, 2–5 cm. long, 8–15 mm. wide, pinnatifid into several pairs of unequal linear lobes 3–7 mm. long, basal leaves merely coarsely sinuate-dentate; petioles very short; flowers few, solitary, sessile in upper axils; calyx-tube 3–5 cm. long, strigulose without, pubescent within at very base; calyx-lobes canescent, coherent at anthesis, lance-linear, 15 mm. long, barely free at tips; petals suborbicular-obovate, reddish, 2–3 cm. long; stamens subequal, the filaments glabrous, scarcely flattened, 10–15 mm. long; anthers ca. 1 cm. long; style exceeding corolla, pubescent at very base; stigma-lobes 5–8 mm. long; capsule oblong-ovoid, canescent, 15 mm. long, ca. 10 mm. thick, with wings ca. 2 mm. wide in the upper half, and with a prominent ridge along middle of each face, dehiscing along upper part of wings; seeds oblong-obovoid, somewhat irregular, quite smooth, brown, 1 mm. long.

Type locality, San Luis Potosi, Mexico. Occurring in northeastern Mexico. Material seen, SAN LUIS POTOSI: San Luis Potosi, *Parry & Palmer 249* (BM, E, G, K, US), *Schaffner 168*, type coll. (G, K), *446* (Ber, BM, Boiss, US), *Barroeta* (US). DURANGO: Durango, *Palmer 966* (US).

3. OENOTHERA HAVARDI Wats., Proc. Amer. Acad. **20**: 366. 1885

Hartmannia Havardii Rose, Contr. U. S. Nat. Herb. **8**: 328. 1905. *H. Palmeri* Rose, l.c., 329.

Perennial with branching caudex and several slender stems 1–2 dm. high, canescent-strigulose to subglabrous, leafy; leaves linear-lanceolate, or the lower ones oblanceolate, 1–3.5 cm. long, 2–5 mm. wide, irregularly

sinuate-pinnatifid, with 3–4 pairs of divergent lobes 2–3 mm. long, basal leaves shorter and merely divaricately dentate; calyx-tube slender, tubular, 4–5 cm. long; calyx-lobes lance-linear, 1.5–2.5 cm. long, coherent in anthesis, without free tips in the bud or with these 1.5 mm. long; petals rhomboid-obovate, 1.7–2.8 cm. long, orange-yellow, turning red; stamens subequal, almost as long as petals; anthers 7–8 mm. long; filaments glabrous, filiform, 9–12 mm. long; style equaling or exceeding corolla; stigma-lobes linear, 2–3 mm. long; capsule oblong-ovoid, 10–15 mm. long, 4–5 mm. thick, "4-angled and the valves strongly ribbed"; seeds brown, minutely cellular-pitted, depressed-obovoid, irregularly angled, 2 mm. long.

Type locality, near Marfa, western Texas. I have seen the following sheets, TEXAS: prairies near Marfa, *Havard 122*, type collection (G, K, US), *Orcutt in 1926* (US). CHIHUAHUA: plains near Chihuahua, *Pringle 1146* (G). DURANGO: Tepehuanes, *Palmer 301* (K, US); Santiago Papasquiaro, *Palmer 45*, type coll. of H. Palmeri (G, K, US). At first it seemed to me that *Hartmannia Palmeri* Rose might be kept as a variety, differing from typical *Havardi* by being glabrous and having free tips to the calyx-lobes. But the three sheets of *Palmer 45* which I have seen are not consistent enough in these characters to warrant recognition.

THE GENUS GAYOPHYTUM

GAYOPHYTUM, A. Juss. in Ann. Sci. Nat. **25**: 18, t. 4. 1832; Benth. & Hooker, Gen. Pl. 1: 786, 789. 1862; T. & G., Fl. N. Am. 1: 512. 1840; Trelease, Report Mo. Bot. Gard. **5**: 107. 1894

Slender-stemmed acaulescent annuals. Leaves alternate, entire, linear, and subsessile, or the lowest may be opposite, linear-oblanceolate, and short-petioled. Flowers in upper axils. Calyx-tube scarcely evident, the calyx being parted to the ovary, the segments often reflexed in anthesis. Petals 4, small, rhomboid-spatulate to -obovate, white, frequently becoming pink or red. Stamens 8, the alternate set much reduced and usually sterile. Stigma capitate. Capsule 2-celled, 4-valved, linear or clavate. Seeds many, in a single row in each cell, not comose.

Type species, *G. humile* Juss.

The genus *Gayophytum* offers an unusually interesting series of plants, varying in a comparatively small number of characters, and these in every conceivable combination. Flowers may be small or large; capsules may be sessile or pedicelled, erect or spreading-deflexed, short or long, torulose or not torulose; the minute hairs may be appressed or spreading; seeds may be glabrous or pubescent; branching may be basal or distinctly above the base. The attempt at classification presented in this paper may be quite artificial; it has been arrived at only after much study. Unfortunately geographical distribution, which is so often of great aid to the systematist, is not very useful here, and one almost doubts the validity of some of the entities maintained because of their lack of continuous or definite distribution. Furthermore, the floral parts are so minute that individual specimens can very easily be thrown into the wrong group. Yet, as I have worked over

many hundreds of sheets at various times during the past three years, I have been forced to conclude that there are several very real and definite entities in the genus. This paper attempts to present my present conception of them.

Key to Species

Capsule torulose, pedicelled; plants freely branched above the base, repeatedly dichotomous, the upper leaves bractlike.
- Seeds glabrous.
  - Plant prostrate, purplish; petals white, 1 mm. long; capsules quite sessile, with longitudinal rows of 2–3 seeds only. So. America ..... 1. *G. micranthum*
  - Plant erect, greenish; petals soon becoming pink to red; capsules pedicelled, with more seeds per row. Mostly No. American.
    - Petals 0.5–1.5 mm. long.
      - Petals 0.5 mm. long; capsule 2–5 mm. long, shorter than the deflexed pedicel; plants quite glabrous ..... 2. *G. ramosissimum*
      - Petals 1–1.5 mm. long; capsule 5–12 mm. long, exceeding the pedicel. 3. *G. Nuttallii*
    - Petals 2–4 mm. long ..... 4. *G. diffusum*
- Seeds appressed-canescent.
  - Petals 1–2 mm. long ..... 5. *G. lasiospermum*
  - Petals 3–4 mm. long ..... 6. *G. eriospermum*

Capsule not torulose, subsessile; plants branched mostly at the base, not so much above; upper leaves quite well developed.
- Seeds vertically placed in a very narrow capsule.
  - Seeds glabrous ..... 7. *G. racemosum*
  - Seeds appressed-canescent ..... 8. *G. Helleri*
- Seeds obliquely placed in a slightly broader capsule ..... 9. *G. humile*

Treatment of Species

1. Gayophytum micranthum (Presl) H. & A. in Hook., Bot. Misc. 3: 311. 1833

Oenothera micrantha Presl, Rel. Haenk. **2**: 31. 1827. *Holostigma paradoxum* Spach, Nouv. Ann. Mus. Paris **4**: 334. 1835. *Sphaerostigma paradoxum* Gay, Fl. Chil. **2**: 329. 1846. *Chamissonia paradoxa* Reiche, Anal. Univ. Chile **98**: 482. 1897. *Gayophytum gracile* Phil., Anal. Univ. Chile **84**: 628. 1893. *G. humile* var. *gracile* Reiche, l.c., 484. *Oenothera gracilis* Lévl., Mon. Onoth., 167. 1905. *Oe. rigida* Gillies, in syn., H. & A., l.c.

Freely branched, with prostrate or laxly ascending stems, commonly 1–2 dm. high, purplish, quite glabrous, somewhat strigulose in upper parts; leaves remote, lance-linear, 5–25 mm. long, 1–2 mm. wide, quite entire, sessile or the lower short-petioled; calyx-lobes 0.5–0.8 mm. long; petals 1 mm. long, white; pedicels 0.5 mm. long; capsule torulose, ascending, 5–6 mm. long, with ca. 3 seeds in each row, purplish; seeds glabrous, 1.5 mm. long.

Type locality, "in regno Chilensi." Confined to South America. Representative material, Chile: without locality, *Oenothera micrantha* Presl, Rel. Haenk. (Ber, and German Univ. at Prague); Andes Chilenses, *Cuming 321*

(BM, Del, K); without locality, *Gay* as *Sphaerostigma paradoxum* (Ber, Del, K, Par); Cord., Chili, *Philippi*, as Graciophylo gracilis (Ber, K); Los Morros, Andes of Mendoza, *Oe. rigida* n. sp., *Gillies* (K); Coquimbo, Baños del Toro, *Werdermann 200* (Ber, BM, G, K); Laguna Valeriano, Atacama, *Johnston 6053* (G, P, K); Uspallata Pass, Juncal, *Buchtien in 1903* (Ber, BM). ARGENTINA: Quebrada Ortega, No. West San Juan, *Johnston 6173* (G, K, P); Penitente Thal, *Gussfeldt in 1883* (Ber).

2. GAYOPHYTUM RAMOSISSIMUM T. & G., Fl. N. Am. 1: 513. 1840

Gayophytum ramosissimum var. *deflexum* Hook., London Journ. Bot. 6: 224. 1847. *Oenothera Gayophytum* race *Treleasiana* f. *ramosissima* Lévl., Mon. Onoth., 169. 1905, in part. *Gayophytum ramosissimum* var. *obtusum* Jeps., Man. Fl. Pls. Calif., 688. 1925.

Diffusely branched, mostly above the base, with the ultimate branches filiform, quite glabrous, sometimes slightly strigulose about the flowers; plant 2–5 dm. tall; leaves lance-linear, 1–3 cm. long, short-petioled, gradually reduced up the stem; pedicels capillary, 3–5 mm. long, mostly spreading-deflexed; flowers minute; calyx-lobes erect, 0.5 mm. long; petals 0.5 mm. long; stigma globose; capsule plump, 2–5 mm. long; seeds glabrous, 0.6 mm. long.

Type locality, "Rocky Mountains," Blackfoot River; type collected by Nuttall. This species is characterized by its minute flowers and short plump capsules on long spreading pedicels, the capsules usually deflexed. Ranging from northeastern California to eastern Washington, then to Montana, Colorado, and Arizona. It is not nearly so common or widespread as the next species. Representative material may be cited as follows, Blackfoot River, Rocky Mts., *Nuttall* (BM, K, Philadelphia Academy); WASHINGTON: Loomis, *Jones in 1911* (P); Washtucna, *Elmer 1042* (Ber, K); Ritzville, *Leiberg & Sandberg 159* (Ber, Par, G, K). OREGON: Steins Mts., *Leiberg 2393* (Ber, G, P); between Pineville and Bear Buttes, *Leiberg 327* (G, K, P); Bend, *Peck 9766* (G). CALIFORNIA: Goose Lake Valley, *Mrs. Austin 468* (P); Chat, *Jones in 1897* (P). NEVADA: Carson City, *Baker 966* (Ber, Del, G, P); Cobre, *Jones in 1906* (P). ARIZONA: Flagstaff, *Jones 3958* (Boiss, Del, P); Buckskin Mts., *Jones in 1896* (P). IDAHO: Coeur d'Alene Mts., *Leiberg 1514* (K, P); Weiser, Washington Co., *Jones in 1899* (P). MONTANA: Spanish Basin, Gallatin Co., *Rydberg & Bessey 4573* (Ber, Del, Boiss, G, K); Monida, *Jones in 1922* (P). WYOMING: Nowood Creek, Big Horn Co., *Goodding 510* (Boiss, Del, G, P); Gardiner River, *A. & E. Nelson 5984* (Ber, Del, G, P, K). COLORADO: Black Canyon, *Baker 192* (Ber, Del, G, K, P); Pagosa Springs, *Baker 493* (Ber, Boiss, Del, G, K, P); Golden, *Greene in 1870* (G). UTAH: Frisco, *Jones 1953* (Ber, Boiss, P); Marysvale, *Jones 5410f* (P).

3. GAYOPHYTUM NUTTALLII T. & G., Fl. N. Am. 1: 514. 1840

With habit and stature of *G. ramosissimum*, usually more evidently puberulent in upper parts; pedicels 1–3(5) mm. long; calyx-lobes 1–1.5 mm. long, reflexed in anthesis; petals reddish at least in age, 1–1.5 mm. long; capsule 5–12 mm. long, usually exceeding the pedicel; seeds glabrous, 1–1.5 mm. long.

More common and widespread than *G. ramosissimum* and often confused with it, but quite distinct in its large flowers, longer capsules and larger seeds. It intergrades freely with *ramosissimum* and *diffusum*.

KEY TO VARIETIES

Pubescence appressed.
Capsules erect ..... 3*a*. *G. Nuttallii* var. *typicum*
Capsules deflexed ..... 3*b*. *G. Nuttallii* var. *intermedium*
Pubescence spreading; capsule erect ..... 3*c*. *G. Nuttallii* var. *Abramsii*

3*a*. **Gayophytum Nuttallii** T. & G. var. **typicum** Munz, n. nom.

G. Nuttallii T. & G., l.c. *G. ramosissimum* var. *strictipes* Hook., London Jour. Bot. 6: 224. 1847. *G. robustum* Phil., Anal. Univ. Chile 84: 628. 1893. *G. humile* var. *robustum* Reiche, Anal. Univ. Chile 98: 484. 1897. *Oenothera micrantha* Nutt., in syn., T. & G., l.c. *Oe. Gayophytum* race *Philippiana* f. *robusta* Lévl., Mon. Onoth., 169. 1905. *Gayophytum ramosissimum* var. *pygmeum* Jeps., Man. Fl. Pls. Calif., 689. 1925.

Pedicels and fruit erect; puberulence appressed.

Type locality, "Rocky Mountains." Ranging from Southern California to Washington, South Dakota, New Mexico; also in Chile and Argentine. In California material the capsules tend to be shorter and fewer seeded than in plants from the rest of the range. *Typicum* intergrades freely with the other varieties. Representative material may be cited, Rocky Mts., *Nuttall* as *Oe. micrantha*, type coll. (BM, K). WASHINGTON: Falcon Valley, *Suksdorf 2257* (Boiss, G); Stehekin, Chelan Lake, *Jones in 1911* (P); Mt. Paddo, *Suksdorf 2296* (Boiss, G). IDAHO: Cedar Mts., Latah Co., *Piper 1633* (G, P). OREGON: Beaver Run, Klamath Co., *Leiberg 615* (G, K, P); Paulina Lake, Crook Co., *Peck 9646* (Del, G). CALIFORNIA: Soda Springs, *Jones 2724* (Ber, P); Hacketts Meadow, Tulare Co., *Culbertson 4466* (G, NY, P); Yosemite, *Abrams 4543* (G, P); Mt. Wilson, Los Angeles Co., *Abrams 2581* (G, K, P); Griffins, Ventura Co., *Elmer 3976* (Ber, G, Del, K, P); Cuyamaca Lake, San Diego Co., *Munz & Harwood 7271* (P). NEVADA: Lake Tahoe, *Hillman in 1894* (P); Charleston Mts., *Jaeger in 1926* (P). UTAH: Alta, *Jones 1148*, in part (Ber, P). MONTANA: Anaconda, *Blankinship in 1906* (P); Midvale, *Umbach 542* (NY). SOUTH DAKOTA: Rochford, *Rydberg 700* (Ber, G). WYOMING: West De Lacy Creek, *A. & E. Nelson 6304* (G, NY); Cora, Sublette Co., *Payson 4344* (G). COLORADO: Elk River, Routt Co., *Goodding 1665* (Ber, Del, G); Mancos, *Baker, Earle*

& *Tracy 389* (Ber, Del, G, K, P). CHILE: Valle hermoso, *Ortega in 1872*, type of *robustum* at Santiago (photo at G, P); Cordillera, sent by Philippi as robustum (Ber, G, K); Paso Cruz, *Kuntze* (Boiss); Cor. de Maule, *Germain in 1855* (Boiss, Del); Cord. de Linares, *Reiche in 1897* (Ber); without locality, *Bridges 1235* (Ber, K). ARGENTINE: Rio Salado, Los Molles, Mendoza, *Kurtz 7463* (Ber).

3*b*. **Gayophytum Nuttallii** T. & G. var. **intermedium** (Rydb.) Munz, n. comb.

G. intermedium Rydb., Bull. Torrey Bot. Club 31: 569. 1904.

Pedicels and fruit spreading or deflexed; puberulence appressed.

Type locality, Canyons and adjoining meadows, west of Ouray, Colorado. This variety ranges from eastern Oregon and Washington to Montana and Colorado. It resembles *G. ramosissimum* in its tendency toward deflexed fruit, but has the long capsules and short pedicels of *Nuttallii*. Representative material is as follows, WASHINGTON: Mt. Paddo, *Suksdorf 2297* (Boiss, NY). IDAHO: Spokane River, Kootenai Co., *Sandberg, MacDougal & Heller 652* (Ber, P). OREGON: Upper Emigrant Creek, Harney Co., *Peck 5620* (G); Mann Lake, *Leiberg 2406* (G). WYOMING: Alpine, Lincoln Co., *Payson & Armstrong 3400* (G, P); Bridger Peak, Carbon Co., *Goodding 2004* (Ber, Del, G). COLORADO: Between Sunshine and Ward, *Tweedy 5092* (NY); Granite, *Harper in 1886* (P); W. of Ouray, *Underwood & Selby 193*, type of intermedium (NY). UTAH: Big Cottonwood Canyon, *Garrett 1649* (G); Mt. Timpanogos, *Garrett 5603* (P).

3*c*. **Gayophytum Nuttallii** T. & G. var. **Abramsii** Munz, n. var.

Plant with short spreading puberulence; pedicels and capsules mostly erect. (Planta breve porrecteque puberula; pedicellis capsulisque fere erectis.)

Type, Coldwater Canyon, San Antonio Mts., Los Angeles Co., California, *Abrams 2694*, July 13, 1920, Pomona College Herbarium No. 156322; isotypes at Gray and Stanford. Ranging from southern California to Washington and Montana. Material referred here has passed as *G. caesium*, but has the torulose capsules of *G. Nuttallii*. Representative material is as follows, WASHINGTON: Falcon Valley, *Suksdorf in 1881* (Boiss, G); Mt. Paddo, *Suksdorf 2295* (Boiss, G); Mt. Adams, *Suksdorf 22* (G); Wenatchee Valley, *Sandberg & Leiberg 528* (G). OREGON: Farewell Bend, Crook Co., *Leiberg 434* (G, P); Mt. Hood, *Jones in 1897* (P). CALIFORNIA: Siskiyou Mts., *T. S. Brandegee in 1885* (G); Shuteye Mt., Madera Co., *Murdoch 2524* (G); Giant Forest, *K. Brandegee* (P); Tuolumne, *Bolander in 1866* (G); Tioga Road, *Smiley 903* (G); Yosemite Valley, *Abrams 4613* (G); Barton Flats, San Bernardino Mts., *Munz & Johnston 8665* (P); Idyllwild, San Jacinto Mts., *Spencer 703* (P). NEVADA: Galena Ranch, Washoe Co., *Kennedy 1292* (NY). IDAHO: Rush Creek, Washington Co., *Jones in 1899*, in part (P). MONTANA: Alta, *Jones in 1909* (P). A collection from Payette Lake, Ida., *Jones in 1899* (P) has reflexed fruits.

4. GAYOPHYTUM DIFFUSUM T. & G., Fl. N. Am. 1: 513. 1840

With general habit and stature of *G. Nuttallii;* pedicels 2–8 mm. long, erect or divaricate; calyx-lobes 2–3 mm. long, reflexed in anthesis; petals white to pink, 2–4 mm. long; stigma clavate-capitate; capsules 5–12 mm. long, divaricate; seed smooth, 1–1.25 mm. long.

KEY TO VARIETIES

Pubescence appressed ..... 4*a*. *G. diffusum* var. *typicum*
Pubescence spreading ..... 4*b*. *G. diffusum* var. *villosum*

4*a*. **Gayophytum diffusum** T. & G. var. **typicum** n. nom.

G. diffusum T. & G., l.c. *Oenothera Gayophytum* race *Treleasiana* f. *diffusa* Lévl., Mon. Onoth., 169. 1905.

Upper parts of plants strigulose.

Type locality, "Rocky Mountains and plains of Oregon." Ranging from southern California to Washington, Montana and Wyoming. Representative collections, WASHINGTON: Loomiston, Okanogan Co., *Elmer 609* (Ber, K, P); Tchimakaine, Spokane Country, *Geyer 546* in part (Ber, G, K); Lake Calispel, *Kreager 345* (G). Without definite locality, *Nuttall*, type coll. (BM, G, K). OREGON: Farewell Bend, *Leiberg 435* in part (G). CALIFORNIA: Goosenest Foothills, Siskiyou Co., *Butler 1630* (P); Morgans Springs, Lassen Butte region, *Eastwood 1817* (G, K); Fosters, Amador Co., *Hansen 503* (Ber, Boiss, Del, K, Par); Mission Pine, Ventura Co., *Hoffmann in 1930* (P). NEVADA: Clear Creek, Canyon, Ormsby Co., *Baker 1246* (Del, G, P). IDAHO: Silver City, Owyhee Co., *Macbride 372* (Del, G, Par); Ketchum, Blaine Co., *Nelson & Macbride 1225* (Del, G, P). WYOMING: Upper Geyser Basin, *A. & E. Nelson 6260* (Ber, Del, G, P). MONTANA: Old Hollowtop, near Pony, *Rydberg 4580* (K, G). Intergrading freely with *G. Nuttallii* in flower size.

4*b*. **Gayophytum diffusum** T. & G. var. **villosum** Munz, n. var.

Pubescence of upper parts of plant of very short, spreading hairs. (Planta breve porrecteque puberulenta in partibus superioribus.)

Type, Farewell Bend, Crook County, Oregon, *Leiberg 435*, Pomona College Herbarium No. 38658. The name *villosum* is an old herbarium name on sheets of the Lyall collection, but without authority and seems never to have been published. This variety is rather rare, and with the range of *typicum*. Material seen, Columbia River, from lat. 46–49 N., *Lyall in 1860* (G, K, Par); near Colville, Columbia River, *Lyall in 1860* (Ber, K); Des Chutes, Ore., *Leiberg 2617* (G); Cimcoe Mts., Wash., *Howell in 1880* (Ber, Del); Falcon Valley, Wash., *Howell in 1882*, in part (Ber, Del); Trinity, Elmore Co., Ida., *Macbride 557* (Del, Par); Mts. California, *Bridges 79* (K); Siskiyou Co., California, *Pringle in 1882* (K); Bear Valley, southern Calif., *Jones in 1900*, in part (P).

5. GAYOPHYTUM LASIOSPERMUM Greene, Pittonia 2: 164. 1891

Plant 2–5 dm. high, branching freely above; leaves linear to lance-linear, 1–3 cm. long, entire; pedicels 3–6 mm. long, divaricate to spreading or even reflexed; calyx-lobes 1 mm. long, reflexed in anthesis; petals white, turning to rose, 1 mm. long; stigma capitate; capsule 4–8 mm. long, torulose; seeds strigose-canescent, 1 mm. long.

KEY TO VARIETIES

Puberulence appressed ..... 5*a*. *G. lasiospermum* var. *typicum*
Puberulence spreading ..... 5*b*. *G. lasiospermum* var. *Hoffmannii*

5*a*. **Gayophytum lasiospermum** Greene var. **typicum** n. nom.

G. lasiospermum Greene, l.c. *Oenothera Gayophytum* race *Treleasiana* f. *lasiosperma* Lévl., Mon. Onoth., 169. 1905.

Puberulence of upper parts of plant closely appressed.

Type locality, "near Julian, San Diego Co., California." Ranging to Washington and Montana. Representative material, WASHINGTON: Junction Crab & Wilson Creeks, Douglas Co., *Sandberg & Leiberg 252* (Ber, K, Par); Rock Island, Kittitas Co., *Sandberg & Leiberg 428* (Ber, G, K, Par); Chelan, *Elmer 488* (Ber, Del, P). OREGON: Mann Lake, *Leiberg 2406* (Ber, K); Keno, Klamath Co., *Peck 9415* (P); Swan Lake Valley, *Applegate 203* (G). CALIFORNIA: North Baldy, San Gabriel Mts., *Abrams & McGregor 583* (G); Palomar Mts., San Diego Co., *Spencer 983* (G, P, Par); Glen Alpine Canyon, *Abrams 12699* (P). NEVADA: Kings Canyon, Ormsby Co., *Baker 1406* (Ber, Del, G, P). IDAHO: Tamarack, Washington Co., *Clark 178* (Del, G, P); Pocatello, *South 296* (P). WYOMING: Sheep Canyon, *Bartholomew 8328* (P). MONTANA: Gallatin Basin, *Blankinship 22*, in part (Boiss). Some collections, cited above, such as *Sandberg & Leiberg 428* approach var. *Hoffmannii* in pubescence. Capsules are occasionally deflexed, as in *Clark 178*.

5*b*. **Gayophytum lasiospermum** Greene var. **Hoffmannii** Munz, n. var.

The puberulence of short spreading hairs. (Planta breve porrecteque puberulenta.)

Type, Stauffer Postoffice, Mt. Pinos, Ventura Co., *Hoffmann in June 27, 1928*, Pomona College Herbarium No. 149730. Occasional, with the variety *typicum*. Material seen, OREGON: Horsethief Meadows, east of Mt. Hood, *Henderson 827* (G). CALIFORNIA: Mt. Eddy, Siskiyou Co., *Heller 11738* (G); Mt. Shasta, *Pringle in 1882* (G); Lake Tahoe, *Hillman in 1894* (P); Tehachapi Mts., *Abrams & McGregor 455* (G); Bluff Lake, San Bernardino Mts., *Munz 10728* (P).

6. GAYOPHYTUM ERIOSPERMUM Coville, Contr. U. S. Nat. Herb. 4: 103. 1893

G. lasiospermum var. *eriospermum* Jeps., Man. Fl. Pls. Calif., 689. 1925.

Like *G. lasiospermum* in stature, habit, and pubescence of seeds; pedicels 4–8 mm. long; calyx-lobes 3 mm. long; petals 3–5 mm. long.

Type locality, east fork of Kaweah River, Tulare Co., Calif. Doubtfully distinct from *G. lasiospermum*, but bearing the same relation to it, as does *G. diffusum* to *G. Nuttallii*. Rare, extending from the southern Sierras in California to Oregon and Idaho. Material seen, CALIFORNIA: Kern River Flat, *Culbertson 4308* (P); Mud Springs, Amador Co., *Hansen 702* (Del); Prattville, *Jones in 1897* (P); dry woods, Siskiyou Co., *Pringle in 1882* (Boiss); Butte Meadows, Butte Co., *Heller 11583* (Del, G). IDAHO: Indian Valley, *Jones 6315* (P); Boise, *Clark 340* (P). OREGON: without locality, *Engelmann* (Ber). Some of the above, as *Culbertson 4308*, *Clark 340*, and *Jones 6315*, have fruits like those of *G. ramosissimum*, while the Jones plant from Prattville has those of *Nuttallii*.

7. GAYOPHYTUM RACEMOSUM T. & G., Fl. N. Am. 1: 514. 1840

Oenothera Gayophytum race *Philippiana* f. *caesia* Lévl., Mon. Onoth., 169. 1905.

Plants low, 1–2 dm. high, subsimple to repeatedly branched from base, the ultimate branches leafy and relatively simple; leaves linear to linear-oblanceolate, 1–3 cm. long; pedicels from almost none to 2 mm. long, erect; calyx-lobes 0.5 mm. long; petals white, turning red, scarcely 1 mm. long; capsule subterete, narrowly linear, not torulose, erect, 6–14 mm. long; seeds erect, glabrous, 1 mm. long.

Plants referred here are often difficult to distinguish from *G. Nuttallii*, which is scarcely leafy in the upper parts and has the capsule more clavate and torulose, and from *G. humile*, which has the seeds oblique.

KEY TO VARIETIES

Plant strigulose or subglabrous ..... 7*a*. *G. racemosum* var. *typicum*
Plant with short spreading hairs ..... 7*b*. *G. racemosum* var. *caesium*

7*a*. **Gayophytum racemosum** T. & G. var. **typicum** n. nom.

G. racemosum T. & G., l.c.

Lower parts glabrous, the upper strigulose or subglabrous.

Type locality, "Near Black-Foot River," Idaho. Occasional from southern California to Washington, Montana, Colorado, and Arizona. Intergrading in pubescence with var. *caesium*. Representative material of var. *typicum:* without locality definitely given, *Nuttall*, type coll. (BM, G, K). WASHINGTON: Mt. Paddo, *Suksdorf 5722* (G); Klickitat River, *Suksdorf 82* (Ber, Boiss); Falcon Valley, *Suksdorf 342* (Ber, Par). OREGON: Steins Mts., *Leiberg 2470* (Ber in part, G, K); Big Meadows, Des Chutes River, *Leiberg 502* (G, P). CALIFORNIA: Davis Creek, Modoc Co., *Black*

in 1894 (K); Chilcott, Plumas Co., *Eastwood 14934* (P); Armstrongs Station, Amador Co., *Hansen 1091* (Boiss, K, Par); Bear Valley, San Bernardino Mts., *Abrams 2901* (Del, G, K, P, Par). ARIZONA: Flagstaff, *Jones 4060* (Boiss, Del, P). UTAH: Alta, *Jones 1249* (Ber, G, P); Cottonwood Canyon, *Garrett 1540* (G, NY). NEVADA: East Humboldt Mts., *Watson 402* (G), *Jones in 1902* (P). MONTANA: Monida, *Jones 8397* (P). WYOMING: Nash's Fork, Albany Co., *Nelson 7793* (Ber, G, K, P). COLORADO: Empire, *Patterson 207* (Del, G).

7*b*. **Gayophytum racemosum** T. & G. var. **caesium** (T. & G.) Munz, n. comb.

G. caesium T. & G., Fl. N. Am. 1: 514. 1840.

Whole plant, or at least the upper portion, with minute short spreading hairs.

Type locality, near Walla Walla, Washington. Occasional from central California to Washington and Nevada. This plant is usually treated as belonging to the *ramosissimum-Nuttallii* group, but has the capsules of *racemosum*. Representative material, near Walla Walla, *Nuttall*, type coll. (BM, G, K). OREGON: Goose Lake Valley, near Lakeview, *Peck 15298* (K); Steins Mts., *Leiberg 2543* (Ber, G). CALIFORNIA: Echo Camp, Eldorado Co., *Heller 12191* (Del, G in part); Green Horn Mts., Kern Co., *Palmer 156* (Boiss, K, Par). NEVADA: Aurum, *Jones in 1893* (Boiss, P); Reno, *Jones in 1897* (P).

8. GAYOPHYTUM HELLERI Rydb., Bull. Torrey Bot. Club 40: 65. 1913

With habit, size and aspect of *G. racemosum*, but with the seeds appressed-canescent.

KEY TO VARIETIES

Plant with short spreading hairs ..... 8*a*. *G. Helleri* var. *typicum*
Plant strigulose or subglabrous ..... 8*b*. *G. Helleri* var. *glabrum*

8*a*. **Gayophytum Helleri** Rydb. var. **typicum** n. nom.

G. Helleri Rydb., l.c. *G. Helleri* var. *erosulatum* Jeps., Man. Fl. Pls. Calif. 689. 1925.

Plant with short spreading hairs.

Type locality, Forest, Nez Perces Co., Idaho. Occasional from southern California to Washington and Idaho. Representative material, WASHINGTON: Rock Island, Kittitas Co., *Sandberg & Leiberg 428* (G). IDAHO: Tamarack, Washington Co., *Clark 187* (Del, G); Shoshone, Lincoln Co., *Nelson & Macbride 1173* (Del, G, NY, P); Forest, Nez Perces Co., type coll., *Heller 3433* (Boiss, NY); Lava Lake, Blaine Co., *Macbride & Payson 3032* (G, P in part). OREGON: west of Harney, Riley Co., *Peck 13850* (K). NEVADA: Kings Canyon, Ormsby Co., *Baker 1042* (Del, G, P). CALIFORNIA: North Baldy, San Gabriel Mts., *Abrams & McGregor 572* (G, Del).

8*b*. **Gayophytum Helleri** Rydb. var. **glabrum** Munz, n. var.

Plant quite glabrous (Planta fere glabra).

Type, Silver City, Owyhee Co., Idaho, *J. F. Macbride 398*, Gray Herbarium; isotype at Delessert Herbarium. Occasional, from central California to Washington, Idaho, Colorado. Representative material is as follows, WASHINGTON: Jct. Crab & Wilson Creeks, Douglas Co., *Sandberg & Leiberg 252* (G). OREGON: Pass through Steins Mts. from Anderson Valley, *Leiberg 2397* (Ber, G, K, Par); Narrows, Harney Co., *Peck 4687* (G). CALIFORNIA: Benton Station, *Jones in 1927* (P). IDAHO: Goose Creek, *Jones in 1899* (P). NEVADA: Carson City, *Jones in 1897* (P); Star Peak, *Jones in 1901* (P). COLORADO: La Sal Creek, *Payson 430* (G).

9. GAYOPHYTUM HUMILE Juss., Ann. Sci. Nat., ser. 1, **25**: 18, t. 4. 1832

Low, 5–15 cm. high, branched from base, the branches relatively simple; leaves linear to lance-linear, 1–3 cm. long, entire, on short petioles, upper ones somewhat reduced, but quite well developed; pedicels scarcely evident; calyx-lobes 1 mm. long; petals white, 1 mm. long; capsule flattened, nontorulose, erect, 10–15 mm. long; seeds obliquely placed in capsules, 0.6 mm. long.

KEY TO VARIETIES

Plant quite glabrous ..... 9*a*. *G. humile* var. *typicum*
Plant with short spreading hairs ..... 9*b*. *G. humile* var. *hirtellum*

9*a*. **Gayophytum humile** Juss. var. **typicum** n. nom.

G. humile Juss., l.c. *G. pumilum* S. Wats., Proc. Am. Acad. **18**: 193. 1883. *G. densifolium* Phil., Anal. Univ. Chile **84**: 629. 1893. *G. humile* var. *densifolium* Reiche, Anal. Univ. Chile **98**: 484. 1897. *G. minutum* Phil., Linnaea **28**: 687. 1856. *Oenothera Gayophytum* race *Philippiana* f. *densifolia* Lévl., Mon. Onoth., 169. 1905.

Plant quite glabrous.

Type locality, Chile. Occasional, Chile; and from southern California to Washington and Idaho. Material from North and South America cannot be separated. Representative collections, CHILE: without locality, *Bridges 1234* (BM, K, Par); Cord. de las Aranas, *Landbeck in 1861*, at Santiago herbarium (photo at G, P); Cord. de Santiago, sent by *Philippi* (Ber, G, K); Cordill., Chili, sent by *Philippi* as densifolium (Ber, K); Andes de Talca, Cajon de Calabozo, type of densifolium, *Philippi in 1879*, at Santiago Herbarium (photo at G, P), specimen sent by Philippi (BM); Cordill. de St. Jago, *Philippi 796* (BM). CALIFORNIA: Yosemite, *Abrams 4586* (G); below Cisco, Placer Co., *Heller 12716* (Del, G); Truckee, *Heller 7057* (Ber, Del, G, P); head of Sacramento River, *Pringle in 1882* (Boiss, G); Lake Co., *Torrey 97*, type of pumilum (G). OREGON: Ashland Butte, Siskiyou Mts., *Howell in 1887* (Boiss, Del); Blue Mts., *Cusick 1737a* (G). IDAHO: Cedar Mts., Latah Co., *Elmer 788* (Ber, Del); Lake Waha, Nez Perces Co., *Heller 3356* (Del, Boiss, Par). WASHINGTON: Falcon Valley, Klickitat Co., *Suksdorf 19* (G).

9*b*. **Gayophytum humile** Juss. var. **hirtellum** Munz, n. var.

Plant puberulent with short spreading hairs. (Planta breve porrecteque puberulenta.)

Type, Snow Valley, Ormsby Co., Nevada, *C. F. Baker 1373*, Pomona College Herbarium No. 32155; isotypes at Berlin, Boissier, Delessert, Gray, and Paris. The name *hirtellum* is an herbarium name proposed by Greene, but never published. This variety looks like *racemosum* var. *caesium*, but has the oblique seeds of *humile;* it is restricted to western Nevada and adjacent California. Material seen, NEVADA: divide south of Slide Mt., Washoe Co., *Heller 10923* (Del, G, NY). CALIFORNIA: Carson Spur or Twin Lakes, Alpine Co., *Hansen 303* (Boiss, Paris in part); Truckee, *Sonne 112* in part (NY).

EXCLUDED SPECIES

1. *G. strictum* Gray, Proc. Amer. Acad. 7: 340. 1867 = Boisduvalia stricta Greene, Fl. Francisc., 225. 1891.

MISCELLANEOUS CHANGES IN NOMENCLATURE

1. OENOTHERA MICRANTHA Hornem., ex Sprengel, Syst. 2: 228. 1825. With the recent change in the International Rules it now becomes necessary to take up this name for *Oe. hirta* Link (not *Oe. hirta* L., which is a *Jussiaea*); see Munz, Bot. Gaz. 85: 260–265. 1928. The following changes are needed: **Oe. micrantha** Hornem. var. **typica** Munz, n. nom., **Oe. micrantha** var. **Jonesii** (Lévl.) Munz, n. comb., and **Oe. micrantha** var. **exfoliata** (Nels.) Munz, n. comb.

2. **Oenothera deltoides** Torr. & Frem. var. **decumbens** (Parry) Munz, n. comb. This varietal name is an earlier one for *Oe. deltoides* var. *ambigua* (Wats.) Munz, Amer. Jour. Bot. 18: 315. 1931. It was first published as *Oe. albicaulis* Nutt. var. (?) *decumbens* Parry, Amer. Nat. 9: 270. 1875, the type collection being *Parry 63* from St. George, Utah.

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THE CHROMOSOMES OF THE CALIFORNIAN LILIACEAE I

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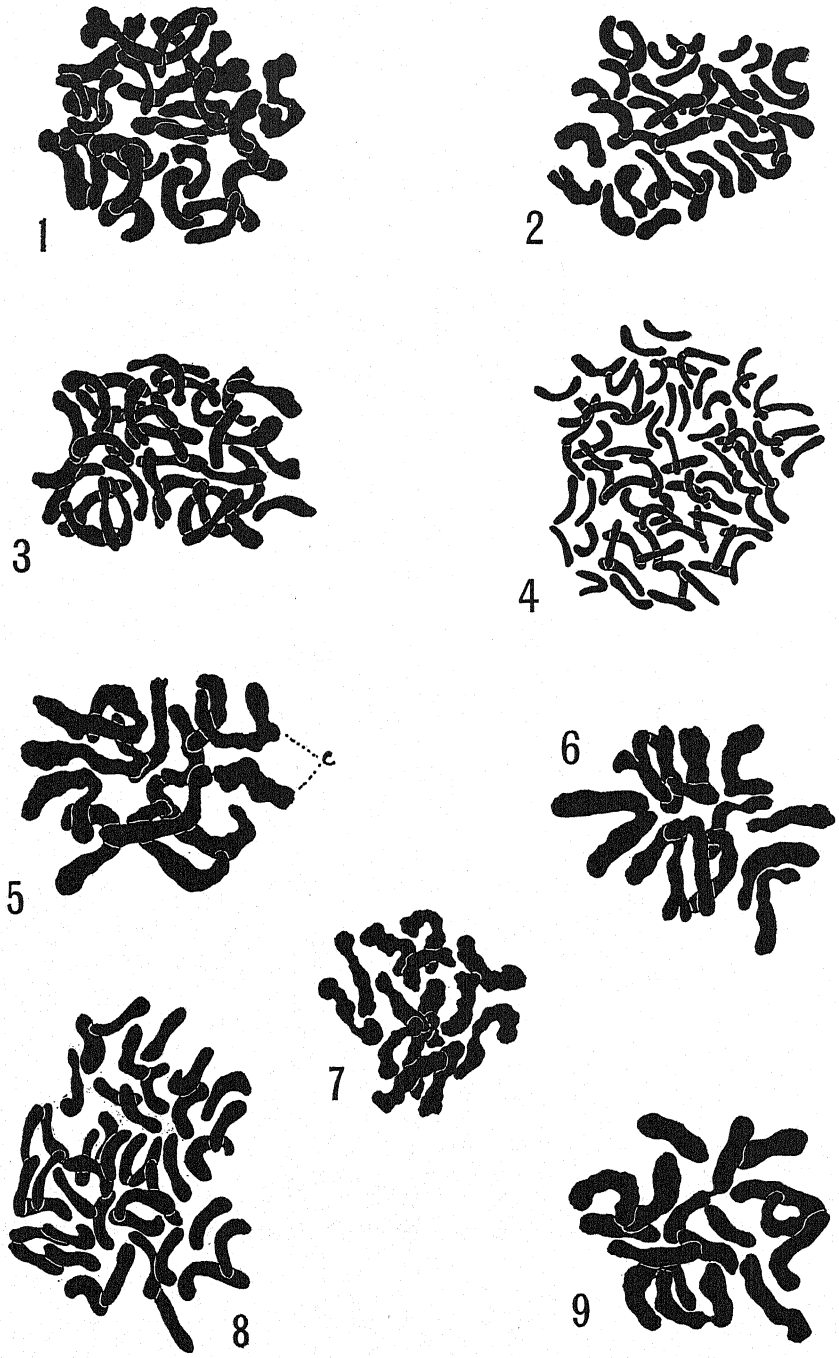
(Received for publication March 17, 1932)

During the past few years, material of the Californian Liliaceae has been collected in the field as opportunity afforded. The immediate objective has been the ascertainment of the chromosomal constitution of each of the known species; while the idea of prosecuting cytomorphological investigations has been kept in mind, it is impracticable to undertake such studies for the time being because of the difficulty of procuring a sufficient quantity of material representing all the stages in the life-history. Phylogenetic considerations for the most part must also be left to the future. Many of the species are rarely found, some are local in distribution, others occur at stations not easily accessible by the ordinary means of transportation; finally, the territory embraced by the state is so vast that it is extremely difficult to complete any large genus that is of statewide distribution. Among such in the Liliaceae might be mentioned *Calochortus, Erythronium, Allium,* and *Triteleia.* Nevertheless, it is now possible to present one genus in its entirety, in addition to one monotypic genus and certain species in other genera which have previously been worked upon by others.

The material investigated was secured either from plants growing in their natural habitats, sometimes from the type locality, or from corms, bulbs, or roots which were collected in the wild and from which root tips were obtained by the sand or water-culture method. It has been deemed necessary to avoid as far as possible using plants procured from commercial growers in order to obviate any danger of contamination by hybridization, as certain of the genera (*e.g., Calochortus*) tend to hybridize freely.

Dr. LeRoy Abrams' *Illustrated Flora of the Pacific States* has been followed almost exclusively in the determination of genera and species. In the author's opinion, the delimitations as given in this manual best follow modern concepts. The general avoidance of such artificialities as varieties and subspecies, which seem absurd to the cytologist, renders it far easier to assign a definite value to the cytological findings. It is rather striking that the results reported below, especially in the cases of *Dichelostemma* and *Hookera,* almost exactly parallel Dr. Abrams' delimitations.

Extremely little has been published on the cytology of the Californian Liliaceae. The only comprehensive paper is that of Newton (1), whose attention was directed principally to *Calochortus.* The species upon which Newton worked are common in cultivation and readily obtainable from any bulb dealer in the state, but he has so obviously taken the trouble to assure himself of the taxonomic and genetic purity of his plants that it is doubtful if



TEXT FIGS. 1–9. Figure 2 is from the outer integument of an ovule; all the others are from root tips. The figures were originally drawn at a magnification of 3900, and have been reduced one-third in reproduction. FIG. 1. *Dichelostemma multiflorum*. FIG. 2. *D. pulchellum*. FIG. 3. *D. californicum*. FIG. 4. *D. capitatum*. FIG. 5. *Hookera californica*. FIG. 6. *H. stellaris*. FIG. 7. *H. minor*. FIG. 8. *H. coronaria*. FIG. 9. *Scoliopus bigelovii*.

material collected in the wild will be found to be any different from material from plants grown in England. The very few other species reported upon will be mentioned under the appropriate genera.

Dichelostemma

The genus *Dichelostemma* Kunth consists of four valid Californian species, all of which have been investigated. A fifth species, *D. pauciflorum* Standl., has been described from Arizona and New Mexico, but it so closely resembles specimens of *D. capitatum* from the desert slopes of southern California that it seems best to follow Dr. Abrams' recommendation (personal discussion) to regard it as merely an inconsequential ecological variant of the latter species. It has not been studied microscopically, no material being available.

D. multiflorum Heller (text fig. 1). The diploid number is 30. The autosomes differ greatly in bulk and in general morphological detail; pairing of homologous chromosomes is fairly clear. In a number of plates there was clear evidence of fragmentation, and in a single plate each member of a large pair had two small satellites. However, I am not wholly convinced that satellites are regularly present.

D. pulchellum Heller (text fig. 2). The only available material was a few ovaries, collected just after fertilization had occurred. Many counts have been made in various ovular tissues, and there seems little doubt that the diploid number is other than 36. There is much difference in the morphology of the chromosomes; pairing in many figures is quite prominent.

D. californicum Wood (text fig. 3). The diploid number, as in the preceding species, is 36. The chromosomes are fairly large, but vary greatly in morphology in different metaphase plates. They are generally thicker at one end and narrowest slightly beyond the center away from the thicker end. This species resembles *D. pulchellum* closely in its cytological aspects, but the two forms are otherwise very different.

D. capitatum Wood (text fig. 4). The diploid number, 72, is an unexpectedly high one, both for the family and for the genus. In order to establish the accuracy of such a high count, more than fifty computations have been made on many root tips from different corms. The chromosomes are essentially similar throughout, although some form differences can be recognized; pairing is fairly distinct. The question arises whether this species might be a tetraploid form of either *D. pulchellum* or *D. californicum,* but the problem does not seem susceptible of ready solution.

Hookera

The genus *Hookera* Salisb. contains eight recognized species, of which four have been examined cytologically.

H. californica Greene (text fig. 5). The sporophytic chromosomes, which total 10, are of a most irregular contour and are very much twisted. It is easy to recognize homologous pairs. One pair is rather peculiarly constructed,

each member being characterized by a deep sub-median constriction. The constricted pair is designated by "*c*" on the accompanying plate.

H. stellaris Greene (text fig. 6). The six pairs of chromosomes, which almost invariably are seen to radiate from a common center, like the spokes of a wheel, are readily distinguished by size and form differences. The autosomes, while much twisted for the most part, are not so strongly convoluted as in the preceding and the following species.

H. minor Britten (text fig. 7). The diploid number is 14. The chromosomes are irregularly thickened and very much convoluted. It is difficult to distinguish homologous pairs because of the lack of size differences, and the twistings render it impossible to make out much of the morphology.

H. coronaria Salisb. (text fig. 8). The diploid number seems high in comparison to the three preceding species, it being 42. The individual chromosomes are somewhat twisted, but tend to have smoother contours as compared to the other forms. Root tip counts were later confirmed by counts in ovular tissues, and in a single first anaphase meiotic figure in a megasporocyte, the haploid number was 21.

Brevoortia

The genus *Brevoortia* Wood contains only one species, although a second has been described. However, the latter (*B. venusta* Greene) is indubitably a hybrid between the valid species and *Dichelostemma capitatum;* it is known only from a single station which we have been unable to relocate. For a long time, I have had a strong feeling that the valid species, *B. ida-maia* Wood, was as much out of the ordinary cytologically as it is systematically. At first it was presumed to be a hybrid, but after Dr. Abrams had pointed out the impossibility of discovering its presumed parentage in any of the known species, in closely related genera (*Dichelostemma, Hookera,* and *Triteleia*), this presumption was abandoned. Meiosis in the microsporocytes so closely resembles the corresponding phenomena in that remarkable group of Californian plants, whose number is constantly being added to, in which oscillating haploid and diploid chromosome numbers regularly prevail, that *B. ida-maia* may be an addition to this group. The group already includes four of the five species of *Clarkia,* two or three in *Crepis,* one in *Lagophylla,* and doubtless several other species in different genera. In microsporocytic meiosis the chromosomes vary strikingly in number and shape but above all in size. The range in number has not yet been determined, but the larger number of countable plates show close to 20 haploid chromosomes. Upon the availability of more material, a critical study of the species is projected.

Scoliopus

The Pacific Coast genus *Scoliopus* Torr. contains but two species, the Californian representative being *S. bigelovii* Torr. The diploid number in this species is 14 (text fig. 9). The chromosomes of this species differ sharply in morphology; the pairs may be classified as follows: (*a*) one long pair, each

member of which has a median constriction, (*b*) one long U-shaped pair, (*c*) one medium long straight pair, (*d*) one medium long curved pair, (*e*) two short, broad pairs, and (*f*) one short, narrow pair.

TRILLIUM

Trillium chloropetalum Howell has 6 haploid and 12 diploid chromosomes, which brings it into agreement with the counts previously reported by other investigators for the two or three eastern species. The form with maroon flowers, as found in the higher foothills of the eastern side of the Santa Clara Valley, was the one studied. It is hardly likely that the greenish-yellow and white flowered forms are any different cytologically. None of the other Californian species has been available for study.

SUMMARY

1. The chromosome numbers, together with significant details of the chromosome morphology, have been described for all the species of *Dichelostemma,* four species of *Hookera,* the sole species of *Brevoortia,* and for one species each in *Scoliopus* and *Trillium.*

2. *Brevoortia ida-maia* may possibly be a species with oscillating chromosome numbers.

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LITERATURE CITED

1. **Newton, W. F. C.** Chrososome studies in *Tulipa* and some related genera. Jour. Linn. Soc. Bot. **47**: 339–354. 1927.

THE NUMBER AND WEIGHT OF SEEDS PRODUCED BY WEEDS[1]

O. A. STEVENS

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INTRODUCTION

Knowledge of the number of seeds produced by individual weed plants is desirable for studies in crop ecology. The approximate weight of individual seeds is continually of use to the seed analyst in determining and checking the percentages found in samples of crop seeds. The present study was begun to secure educational material. It has been continued for five years in the belief that a comprehensive report would be of value and might permit some generalizations. The term "weed" has been used in its broadest sense and a few cultivated or other wild plants have been included.

METHODS

The literature on weeds contains numerous references to the number of seeds produced by plants of various species. Usually these are without any explanation of the way in which the results were obtained. In the present work a definite method of collecting and cleaning was followed. Except where otherwise indicated, the writer has personally selected the plants and supervised the work throughout. An average, well developed plant, growing with comparatively little competition, was selected at a time when it would have the maximum number of mature seeds. After drying and threshing, the seeds were cleaned and finally recleaned with a vertical air-blast to retain only plump, well developed seeds and, unless otherwise noted, only these are included in the results. The last point is especially important because in many experiments on weed seeds, it appears that the results have been obscured by the use of immature material.

The method of collection is of course not well adapted to use with plants the seeds of which are released as soon as mature. Thus it is almost useless for a great many Asteraceae and Cichoriaceae, and unsatisfactory for *Oxalis, Euphorbia,* and some Fabaceae. Explosive fruits and parachutes are not the only factors which interfere. In several of the large families one finds species which drop their seeds or fruits readily and others which do not. In the Brassicaceae, the pods of *Thlaspi, Conringia, Brassica,* and *Camelina* remain intact until all are well matured. *Bursa* in particular, begins to drop its seeds very early and the plant continues to flower and develop additional fruits for some time. *Lepidium* and many others occupy a somewhat intermediate posi-

[1] Contribution from Department of Botany, North Dakota Agricultural Experiment Station. Published with the approval of the Director.

TABLE 1. *Seed Production of Individual Plants*

Species	Wt. 1000 Seeds (grams)	Total Number	Remarks
	Alismaceae		
Alisma plantago-aquatica L.	.550	21,200	
	Poaceae		
Agropyron tenerum Vasey	3.250	6,630	
Agrostis hyemalis (Walt.) B. S. P.	.043	16,000	
Alopecurus geniculatus L.	.200	5,000*	
Avena fatua L.	17.520	250*	Large plant
Beckmannia erucaeformis (L.) Host	.530	32,700	All glumes present
Beckmannia erucaeformis	.300	—	Without outer glumes
Cenchrus pauciflorus Benth.	6.750	1,110*	Caryopses
Chaetochloa glauca (L.) Scribn.	4.200	6,420*	Large plant
Chaetochloa viridis (L.) Beauv.	1.475	34,000	Large plant
Echinochloa crusgalli (L.) Beauv.	1.400	7,160*,†	
Elymus canadensis L.	5.350	1,200	
Eragrostis cilianensis (All.) Link	.075	82,100	
Eragrostis pilosa (L.) Beauv.	.045	4,200*	Minn.
Hordeum jubatum L.	1.125	2,420	Awns removed
Panicum capillare L.	.650	11,400*	
Panicum virgatum L.	1.275	38*,‡	
Sporobolus asper (Michx.) Kunth	.650	4,540	
Sporobolus cryptandrus (Torr.) Gray	.320	35,800*	
Sporobolus neglectus Nash	.525	5,170†	
Stipa viridula Trin.	3.050	740*	
Syntherisma sanguinalis (L.) Dulac	.575	27,100*	Minn.
Tridens flavus (L.) Hitch	.950	330‡	Pa.
	Cyperaceae		
Carex festucacea Schkuhr	1.300	4,200	
Carex vulpinoidea Michx	.350	19,000	
Cyperus diandrus Torr	.120	1,300	
Cyperus esculentus L.	.190	2,420‡	
Cyperus schweinitzii Torr.	1.400	950*	
Eleocharis engelmanni Steud.	.210	1,785	
Eleocharis palustris (L.) R. & S.	.500	1,920	Tuft 1.5 dm. square
Scirpus americanus Pers.	2.200	80	10 stems
Scirpus atrovirens Muhl.	.070	59,140	8 stems
Scirpus nevadensis S. Wats.	.950	24	3 spikes
Scirpus paludosus A. Nels.	3.250	2,060	6 stems
	Commelinaceae		
Tradescantia occidentalis (Britton) Smyth	3.000	1,160*	
	Juncaceae		
Juncus bufonius L.	.015	5,300	
Juncus torreyi Coville	.012	17,080	
	Liliaceae		
Zygadenus chloranthus Richards	.775	86	
	Iridaceae		
Sisyrinchium angustifolium Mill.	.600		
	Urticaceae		
Urtica gracilis Ait.	.105	26,600‡	
Urticastrum divaricatum (L.) Kuntze	1.300	1,120	

TABLE I—(*Continued*)

Species	Wt. 1000 Seeds (grams)	Total Number	Remarks
	Polygonaceae		
Polygonum aviculare L.	.675	6,380*	Large plant
Polygonum convolvulus L.	7.000	11,900	Large plant
Polygonum erectum L.	2.125	1,360*	
Polygonum hydropiper L.	1.650	3,300	
Polygonum lapathifolium L.	1.550	19,300	
Polygonum pennsylvanicum L.	3.600	3,140*	Minn.
Polygonum persicaria L.	1.775	1,550*	
Polygonum ramosissimum Michx.	4.150	1,500	
Polygonum virginianum L.	5.300	189‡	Pa.
Rumex acetosella L.	.525	250‡	Pa.
Rumex crispus L.	1.400	29,500	
Rumex mexicanus Meisn.	1.075	13,000	
Rumex occidentalis Wats.	.800	23,000	
Rumex persicarioides L.	.150	98,250	
	Chenopodiaceae		
Atriplex argentea Nutt.	7.500	4,300	Incl. bracts
Atriplex hastata L.	.500	13,760*	
Atriplex patula L.	2.150	16,460	
Atriplex rosea L.	3.000	12,830	Incl. bracts
Atriplex rosea	1.400	—	Without bracts
Axyris amaranthoides L.	1.100	11,640	
Chenopodium album L.	.700	72,450	
Chenopodium berlandieri Moq.	.610	40,800*	
Chenopodium gigantospermum Aellen (*C. hybridum* of Am. auth.)	1.000	27,500	
Chenopodium leptophyllum Nutt.	.380	21,500*	
Chenopodium standleyanum Aellen (*C. boscianum* in part)	.700	1,900*	
Chenopodium rubrum L.	.060	176,300	
Corispermum emarginatum Rydb.	1.900	—	
Corispermum nitidum Kit.	1.300	1,110*	
Corispermum villosum Rydb.	1.325	1,800*	
Cycloloma atriplicifolium (Spreng.) Coult.	1.650	26,800	Fruits
Cycloloma atriplicifolium	1.500	18,000	Seeds
Kochia scoparia (L.) Schrad.	.850	14,600	
Monolepis nuttalliana (R. & S.) Wats.	.330	7,900	
Salsola tragus L.	1.675	24,700	
	Amaranthaceae		
Amaranthus blitoides Wats.	.950	14,600*	
Amaranthus graecizans L.	.230	129,000*	
Amaranthus retroflexus L.	.380	117,400*	
Acnida tuberculata Moq.	.190	35,000	
	Nyctaginaceae		
Allionia nyctaginea Michx.	6.350	560*	
	Caryophyllaceae		
Saponaria officinalis L.	1.375	1,950	Mich., 7 stems
Silene antirrhina L.	.100	14,200	
Silene fabaria Sibth. & Sm.	.400	6,100*	
Silene noctiflora L.	.850	1,800	Small plant
Vaccaria vulgaris Host	8.800	4,300	Large plant
	Portulacaceae		
Portulaca oleracea L.	.130	52,300	

TABLE 1—(*Continued*)

Species	Wt. 1000 Seeds (grams)	Total Number	Remarks
	Ranunculaceae		
Anemone canadensis L.	3.250	157‡	4 or 5 fls.
Thalictrum dioicum L.	2.500	1,300‡	
	Brassicaceae		
Arabis hirsuta (L.) Scop.	.060	11,250	
Berteroa incana (L.) DC.	.450	2,530	
Brassica arvensis (L.) Kuntze	1.900	2,700	
Brassica juncea (L.) Cosson	2.625	4,950	
Brassica nigra (L.) Koch	1.700	13,400	
Bursa bursa-pastoris (L.) Britton	.096	38,500*,†	
Cakile edentulata (Bigel.) Hook	34.850	950	Entire pods; Ind.
Cakile edentulata	6.350	—	Seeds
Camelina dentata Pers.	2.650	1,970	
Camelina microcarpa Andrz.	.275	2,100	
Camelina sativa (L.) Crantz	1.000	1,640	
Conringia orientalis (L.) Dumort	2.150	3,800	
Erysimum asperum DC.	.450	1,075	
Erysimum cheiranthoides L.	.125	30,500	
Erysimum parviflorum Nutt.	.180	4,600	
Erucastrum pollichii Schimp. & Spenn.	.410	8,480*	
Hesperis matronalis L.	2.025	5,000	
Lepidium densiflorum Schrad.	.250	6,000	
Lepidium ramosissimum A. Nels.	.220	2,680	
Neslia paniculata (L.) Desv.	3.050	490	Pods
Sisymbrium altissimum L.	.175	80,400*	
Sophia hartwegiana (Fourn.) Greene	.080	12,375	
Sophia intermedia Rydb.	.130	5,700*	
Sophia parviflora (L.) Standl.	.120	75,650	
Thelypodium integrifolium (Nutt.) Endl.	.320	12,200	
Thlaspi arvense L.	.785	7,040	
	Capparidaceae		
Cleome serrulata Pursh	6.150	8,330*	
Polanisia graveolens Raf.	3.050	2,800	
	Penthoraceae		
Penthorum sedoides L.	.010	320,000	
	Rosaceae		
Drymocallis arguta (Pursh) Rydb.	.160	15,150	
Geum canadense Jacq.	1.700	2,240†	
Potentilla millegrana Engelm.	.040	50,000	
Potentilla monspeliensis L.	.130	48,600	
Potentilla paradoxa Nutt.	.120	22,500	
Potentilla recta L.	.200	12,500	N. Y.
Potentilla pennsylvanica strigosa Pursh.	.135	6,380	
Rosa pratincola Greene	18.500	189‡	
Spiraea salicifolia L.	.080	115‡	
	Fabaceae		
Astragalus crassicarpus Nutt.	3.100	1,600	
Astragalus flexuosus Dougl.	1.350	3,040	
Glycyrrhiza lepidota (Nutt.) Pursh	8.620	185‡	
Hosackia americana (Nutt.) Piper	6.000	66*	
Meibomia canadensis (L.) Kuntze	6.200	133	Many weeviled
Medicago lupulina L.	1.200	2,350	
Medicago sativa L.	1.850	5,320	

TABLE 1—(*Continued*)

Species	Wt. 1000 Seeds (grams)	Total Number	Remarks
Melilotus alba Desr	2.000	14,235	5 stems
Oxytropis lambertii Pursh	.875	1,200	
Petalostemon purpureum (Vent.) Rydb.	1.300	700*	
Petalostemon villosum Nutt	1.950	1,015*	
Vicia angustifolia (L.) Reichard	18.200	150	
	Oxalidaceae		
Oxalis cymosa Small	.237	570*	
Oxalis stricta L	.150	570*	
	Linaceae		
Linum lewisii Pursh	1.075	370*	
Linum rigidum Pursh	.700	215*	(Infertile?)
Linum sulcatum Riddell	.300	210	
Linum usitatissimum L	—	300	
	Euphorbiaceae		
Acalypha virginica L	.670	105	Pa.
Euphorbia esula L	3.500	140‡	
Euphorbia nutans Lag	.560	1,720*	Minn.
Euphorbia serpyllifolia Pers	.300	2,670*, †	
Euphorbia humistrata Engelm	.240	14,100	Minn.
	Malvaceae		
Malva borealis Wallm	1.300	47,500	
Malva borealis	2.025	—	Carpels
	Onagraceae		
Ludwigia alternifolia L	.0285	11,500†	Pa.
Oenothera biennis L	.330	118,500	
	Apiaceae (mericarps)		
Cicuta maculata L	1.450	5,500	
Sanicula marylandica L	7.700	110	
Sium cicutaefolium Schrank	.600	14,580	
Washingtonia longistylis (Torr.) Britton	12.000	2,425	
Zizia cordata (Walt.) DC	1.725	3,130	
	Gentianaceae		
Gentiana procera Holm	.050	2,700	3 pods
	Apocynaceae		
Apocynum hypericifolium Ait	.700	1,160‡	
	Asclepiadaceae		
Asclepias syriaca L	4.200	770‡	4 pods
	Convolvulaceae		
Convolvulus repens L	30.400	50‡	
	Cuscutaceae		
Cuscuta arvensis Beyr	.775	16,000†	
Cuscuta cephalanthi Engelm	1.250	1,000	Pa.; 1 host stalk
Cuscuta coryli Engelm	2.250	7,000	
	Polemoniaceae		
Collomia linearis Nutt	.750	175	

TABLE 1—(*Continued*)

Species	Wt. 1000 Seeds (grams)	Total Number	Remarks
Hydrophyllaceae			
Macrocalyx nyctelea (L.) Kuntze	6.150	1,070*,†	
Boraginaceae (nutlets)			
Lappula americana (A. Gray) Rydb	2.425	3,820	
Lappula echinata Gilibert	1.350	2,120*	
Onosmodium occidentale Mack	14.100	1,030	
Verbenaceae (nutlets)			
Verbena bracteosa Michx	.500	10,620	
Verbena hastata L	.320	20,000	
Verbena stricta Vent	.850	3,800	
Verbena urticifolia L	.475	10,740	
Lamiaceae (nutlets)			
Dracocephalum parviflorum Nutt	2.625	49,600	Large plant
Hedeoma hispida Pursh	.270	6,500	
Koellia flexuosa (Walt.) Mac M	.080	2,250‡	Pa.
Leonurus cardiaca L	.775	16,340	
Lycopus americanus Muhl	.150	34,560	
Lycopus lucidus Turcz	.500	600‡	
Mentha canadensis L	.100	5,500‡	
Monarda fistulosa L	.320	767	Many weeviled
Nepeta cataria L	.500	46,940	
Prunella vulgaris L	.600	335	Pa.; 4 spikes
Scutellaria lateriflora L	.400	900 Pa.	
Stachys palustris L	.775	64‡	
Teucrium canadense L	1.750	160‡	Pa.
Teucrium occidentale Gray	.700	900‡	(Large?)
Solanaceae			
Solanum rostratum Dunal	2.300	8,460*	
Solanum triflorum Nutt	1.150	5,775	
Solanum triflorum (stone cells)	.600	750	
Scrophulariaceae			
Castilleja sessiliflora Pursh	.100	4,300	
Gratiola virginiana L	.011	46,300*	
Ilysanthes dubia (L.) Barnh	.0033	—	
Linaria vulgaris Hill	.140	2,280	Pa.; 9 stems
Mimulus ringens L	.010	301,500	
Orthocarpus luteus Nutt	.130	1,150	
Scrophularia leporella Bickn	.140	11,200	
Verbascum thapsus L	.090	223,200	
Veronica peregrina L	.030	77,000	
Plantaginaceae			
Plantago major L	.200	36,150	
Plantago purshii R. & S	.750	1,160	
Plantago rugelii Dec	.700	5,000	
Rubiaceae			
Galium boreale L	.600	1,300	
Caprifoliaceae			
Symphoricarpos occidentalis Hook	5.850	1,100‡	

TABLE I—(*Continued*)

Species	Wt. 1000 Seeds (grams)	Total Number	Remarks
	Ambrosiaceae		
Ambrosia artemisiaefolia L.	3.950	3,380*	
Ambrosia trifida L.	17.400	1,650*	(50%)
Iva axillaris Pursh	2.800	165‡	(40% weeviled)
Iva xanthiifolia Nutt.	1.200	82,150	
Xanthium italicum Mor.	38.000	650	Estimated
Xanthium italicum	200.000	440	Burs
	Asteraceae		
Achillea millefolium L.	.400	210‡	
Arctium minus Bernh.	7.500	31,600	Many weeviled
Artemisia biennis Willd.	.075	1,075,000	
Artemisia caudata Michx.	.100	215,000*	
Bidens acuta (Wieg.) Britton	1.720	1,550	
Bidens frondosa L.	3.500	7,000	
Bidens vulgata Greene	5.100	1,940	
Boltonia asteroides (L.) L'Her.	.220	10,000*	
Brauneria angustifolia (DC.) Heller	2.500	—	
Chrysanthemum leucanthemum L.	.220	510	Pa.; 3 heads
Cirsium arvense (L.) Scop.	1.575	680*,‡	
Cirsium megacephalum (Gray) Cockerell	8.450	119‡	Relatively good
Coreopsis tinctoria Nutt.	.090	5,150	
Eupatorium perfoliatum L.	.170	37,000	Pa.
Grindelia squarrosa (Pursh) Dunal	.600	29,700	Many weeviled
Helenium montanum Nutt.	.310	17,710	Fls. mostly attached
Helenium montanum	.220	—	Fls. removed
Helianthus annuus L.	6.575	7,200*,†	
Helianthus maximiliani Schrad.	2.225	2,600	4 stems
Helianthus petiolaris Nutt.	2.100	3,850*,†	
Helianthus tuberosus L.	6.000	47‡	Many infertile
Heliopsis scabra Dunal.	3.900	2,300	Many weeviled
Matricaria suaveolens (Pursh) Buchenau	.130	850*	
Ratibida columnaris (Sims) D. Don	.440	7,000	
Rudbeckia hirta L.	.260	1,615	Many weeviled
Rudbeckia laciniata L.	1.800	510*	(Infertile ?)
Sideranthus spinulosus (Nutt.) Sweet	.550	—	
Sideranthus spinulosus	.400	—	Rubbed
Solidago rigida L.	.450	3,290‡	Rubbed
Verbesina alternifolia (L.) Britton	3.600	480	Pa.
	Cichoriaceae		
Lactuca scariola L.	.450	27,900	Estimated
Sonchus arvensis L.	.380	9,750‡	62 heads

* Many immature seeds also present.
† Many seeds shattered.
‡ Yield of one main stem.

tion. *Echinochloa* in the Poaceae behaves much like *Bursa*. The data for *Sonchus* were secured by daily collecting for a 30-day period. The method serves well for many species. The figures given in the table of course show only the general magnitude of production. I have estimated roughly that they would be increased twice for a large plant, and twice again if only a part can be secured at one time.

For perennials which spread by roots or rhizomes, a single plant cannot be delimited. The product of a single stem or of a unit area appears to be the only method of comparison. In most cases I have used the former which is fairly satisfactory. For the perennials which grow in clumps I have tried to indicate the number of stems present. Table 1 presents the total number of seeds produced by each species and the weights of 1000 seeds calculated from 200, 500, or 1000 according to size of the seed.

Discussion

A personal equation is involved in the selection of an average sized plant, but it is believed that this has been reduced to a reasonable minimum. As has been suggested in table 1, some of the plants which have been secured were not quite as desired. Other collections yielding results similar to those quoted, were made of *Amaranthus retroflexus, Arabis hirsuta, Euphorbia serpyllifolia, Ambrosia trifida, Helianthus tuberosus,* and *Rudbeckia hirta.* Considerable variation in weight was found in some of the larger seeded species, two other plants of *Vaccaria* giving results of 6.66 and 7.50 g. per 1000 seeds. *Ambrosia trifida* was found to have fruits of distinctive shape on different plants and they varied in weight from 17.40 to 33.40 g. Insects and disease reduce seed production of many species. *Grindelia, Helianthus, Rudbeckia, Arctium, Cirsium, Iva axillaris,* and *Meibomia* were badly weeviled. One plant of *Eragrostis cilianensis* was found to be smutted and *Rumex mexicanus* produces very few fruits as a rule.

If one inquires what class or group of plants produced the largest numbers of seeds, the data presented in this paper may be summarized as follows:

Table 2. *Numbers of Seeds Produced by Various Groups*

Groups	Annuals	Perennials*	Biennials
Poaceae	(10 spp.) 17,891	(9 spp.) 11,640	(no species)
Polygonaceae	(8 spp.) 17,945	(3 spp.) 21,883	(no species)
Chenopodiaceae	(17 spp.) 27,500	(0 spp.) —	(no species)
Brassicaceae	(17 spp.) 15,717	(0 spp.) —	(8 spp.) 8,727
Asteraceae	(8 spp.) 3,816	(6 spp.) 10,833	(5 spp.) 266,263
All species	(101 spp.) 20,832	(61 spp.) 16,629	(21 spp.) 85,500

* Excluding those which spread freely underground.

These results show a remarkable similarity for the three growth groups and the five chief families. If we omit the two species of *Artemisia,* the averages for the biennial Asteraceae and for all biennials become 13,772 and 26,600, respectively. This would leave biennials distinctly in the lead and annuals slightly ahead of perennials. This seems more logical than the averages found by Stone (5) from a smaller number of species in which the proportions were approximately 1–3–8 for perennials, biennials, and annuals, respectively.

Such attempts at generalization seem not especially useful. Perhaps it would be better to place all species in a few groups such as: 1000 seeds or less, 23 species; 1000 to 10,000, 86 species; 10,000 to 50,000, 56 species; 50,000 to 100,000, 9 species; above 100,000, 9 species.

It appears that few species produce more than 100,000 seeds per plant. The biennials have rather more than their share with *Artemisia, Oenothera,* and *Verbascum.*

Korsmo (2) has published seed production data for the common weeds of Europe. In a letter to the writer he states that these represented average plants growing among crop plants. It has seemed more to the point to determine the number which are produced under normal conditions without competition. A few weed plants often remain in a field which has been tilled to destroy weeds. In such a case the degree of success of the cultivation is determined largely by the seed production of these few plants which survive. Stone (5) has given results on a number of species, and in reply to an inquiry regarding the method used, he writes that the average was taken of the smallest, of an average sized, and of the largest plant found. However, the number of seeds produced by the smallest would approach zero, so that figure might be disregarded. The ratio of the average of the numbers found by Korsmo, by the writer, and by Stone from eight species which occur in all three lists is approximately 1–14–192.

Size of seed is rather evidently related to the number produced. The highest total weight per plant was *Arctium* with 237 grams. Size appears rather more variable within families than might have been expected. Poaceae and Cyperaceae show much diversity. Most Polygonaceae have medium sized seeds but a few species have fairly large and a few quite small ones. The seeds of many Brassicaceae are very small, but those of others are fairly large. Asteraceae show a wide range. The Scrophulariaceae are noted for their minute seeds but a considerable range is shown in the few listed and it would be increased by others such as *Pentstemon.*

Size and number of seeds comprise two factors in the success of the plant. The length of time required to complete growth is more important. The biennial plants are an interesting illustration of this point. *Artemisia biennis,* which produces the largest number, also is among the smallest seeded species. It is one of the last plants of the year to come into flowering condition, hence the seedlings must pass through the vicissitudes of two seasons. Biennials have little chance of survival in fields which receive any sort of annual cultivation. They are chiefly weeds of meadows, roadsides and fields left untilled for one or more seasons.

Thlaspi arvense is a remarkably successful weed. Its seeds are produced in only moderate number, but they are of fair size; the seedlings grow rapidly and the plant matures early. Its winter annual habit takes advantage of comparative immunity from cultivation in the spring grain districts, though the seedlings are subject to a considerable degree of winter killing. The seeds

have no natural means of distribution, hence the plants must have a high degree of toleration. Often the seedlings appear in such numbers that they are unable to make appreciable growth. In three different years on square meter areas where there were practically pure stands, I counted 342 and 220 plants (not counted the third year), which yielded 156,000, 107,400, and 125,000 seeds, respectively.

The query may be raised whether the 1000-seed weight does not vary considerably with individual plants. Some data were given in the notes following table 1 which indicate such variation in some of the larger seeded species. For the majority I believe the variation would not be much larger in mass collections than in individual plants. Comparison of the weights in table 1 with those published by Korsmo (2), by Pammel and King (4), and by the Ottawa Laboratory (3) show considerable variation, the weights given by Korsmo running distinctly higher (table 3).

TABLE 3. *Weight of 1000 Seeds as Found by Korsmo, Pammel and King, Ottawa Laboratory, and Stevens*

Species	Weight in Grams of 1000 Seeds as Found by:			
	Korsmo	Pammel	Ottawa	Stevens
Avena fatua	22.500	22.220	21.240	17.500
Chaetochloa glauca	3.100	1.280*	2.235	4.200
Chaetochloa viridis	—	.469	1.148	1.475
Echinochloa crusgalli	1.500	.720*	1.175	1.400
Panicum capillare	—	.380	.349	.650
Syntherisma sanguinalis	—	.270	.325	.575
Polygonum aviculare	2.700	—	1.000	.675
Polygonum convolvulus	5.000	4.650	5.182	7.000
Polygonum hydropiper	2.500	1.100	2.250	1.650
Polygonum lapathifolium	3.600	1.570	.800	1.550
Polygonum persicaria	2.700	1.400	1.177	1.775
Rumex acetosella	.300	5.000†	.450	.525
Rumex crispus	1.400	1.370	1.400	1.400
Chenopodium album	1.150	.690	.701	.700
Amaranthus retroflexus	—	.370	.439	.380
Silene noctiflora	—	1.000	.964	.850
Vaccaria vulgaris	—	4.380	5.912	8.800
Brassica arvensis	1.250	2.020	2.419	1.900
Thlaspi arvense	1.750	—	1.200	.785
Potentilla monspeliensis	—	.084	.100	.130
Arctium minus	11.400	3.120‡	7.700	7.500
Chrysanthemum leucanthemum	.450	—	.295	.220
Cirsium arvense	1.150	1.130	.900	1.575
Sonchus arvensis	.500	—	.485	.380

* Probably immature.
† Doubtless a misprint for .500.
‡ Given as *Arctium lappa*, but surely *A. minus*.

For a few of the European plants, *e.g., Polygonum aviculare* and *Chenopodium album,* there may be a question what European race was considered. I am unable to account, however, for the high weights reported for *Polygonum lapathifolium, P. persicaria,* and *Thlaspi arvense* or for the low one of *Brassica arvensis.* The degree of maturity and of threshing and cleaning probably is

responsible for a large part of the differences. *Chaetochloa glauca* should be noted especially. The Canadian records were from old seed at least in part, but this should not affect the results materially. The Iowa records were stated to be: "so far as possible, seeds of average size and fresh condition."

Weed seeds taken from samples of cleaned crop seed may vary somewhat due to selection in cleaning according to the size of the crop seeds in which they occur. I had collected some data from this source (1) and had noted that *Chaetochloa viridis* taken from red clover weighed 1.093 grams per 1000 seeds, but 1.238 grams when taken from millet. Similarly *Rumex acetosella* weighed .408 and .691 grams per 1000 seeds when taken from alsike and red clover, respectively.

Summary

This paper presents data upon the seed production of common weeds and other plants with particular regard to selection of specimens and cleaning of seed to a definite plan. Comparison of results with those found by other workers and consideration of conditions presented by various species has suggested some explanations for differences in results. A suggestion for further studies is that they might be in the nature of comprehensive investigations of the seed habits of particular species.

The material of species marked "Pa." in table 1 was collected by Mrs. Salome C. Miller near Harrisburg, Pennsylvania. A few specimens received from various localities in North Dakota for identification were used. Acknowledgment is made also to Prof. Emil Korsmo of Oslo, Norway, for information and assistance; to Mr. G. A. Elliott of the Ottawa Laboratory for checking a number of results quoted in table 3; to Prof. A. L. Stone of the University of Wisconsin for information relating to data, and to Dr. Herbert C. Hanson for suggestions in preparation of the manuscript.

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DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND EMBRYO IN COTTON

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The studies reported in this paper were undertaken to clear up some of the conflicting data on the reproductive processes in cotton, especially the development of the female gametophyte and early stages in the embryo.

Guignard (6), in his early work on double fertilization, included in his studies two species of the family Malvaceae. He described the mature female gametophyte, pollen tube growth, and fertilization in *Hibiscus trionum* and *Althaea rosea*. He has reported the following characteristics for *Hibiscus* and *Althaea* which are also common in *Gossypium:* a well developed nucellus, early disappearance of the antipodals, late fusion of the polar nuclei, the persistent synergids, and the formation of several endosperm cells before the division of the zygote. He also reports in *Hibiscus* extensive branching of the pollen tube after its entrance into the nucellus as well as the development of several tubes from one pollen grain.

Balls (1) has described the development of the floral organs, the embryo sac, the microspore, and fertilization in cotton. He also described the early stages of the embryo and early growth of lint hairs. His studies were made on plants grown from commercial Mit-Afifi seed, a variety of Egyptian cotton placed under *G. barbadense*. He reports the functioning of the micropylar megaspore in the development of the female gametophyte and the lack of a suspensor in the embryo in this species.

Denham (4) has studied the reduction divisions in a variety of Sea Island cotton (*Gossypium barbadense* L.). His conclusions are that reduction division takes place along normal telosynaptic lines. He also gives the structure of the mature microspore. In the second paper of this series, Denham (4) reports the chromosome number of thirty-two varieties of cotton including Upland, Sea Island, Egyptian, Indian, and Chinese types. He finds that the Old World cottons have thirteen pairs of chromosomes and that the New World cottons have twenty-six pairs. This difference in chromosome num-

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bers, he explains, may account for the difficulty of securing fertile hybrids when the New World cottons are crossed with the Old World species.

Beal (2) made a study of the heterotypic prophases in reduction division in microsporogenesis, using material of Sea Island, Pima, and Upland cottons. He concludes that the heterotypic pairing in cotton is parasynaptic rather than telosynaptic and gives the haploid chromosome number as twenty-six for the varieties studied.

The economic value of cotton, as well as the wide interest in problems of its heredity, well warrants a careful study of fertilization and closely related processes of our most common cultivated species. Such information may throw some light on the sterility in hybrids between Pima-Egyptian and American Upland cottons as well as the failure of attempts to hybridize these cottons with other related species.

Materials and Methods

In nearly all cases material for these studies was obtained from selfed strains of these three common species of cotton: Sea Island (*Gossypium barbadense* L.), Pima (*G. peruvianum* Cav.), and Upland (*G. hirsutum* L.) of the Delfos 6102 and Mebane varieties. Pima is more often placed with Sea Island under *G. barbadense* L., but it is here placed under *G. peruvianum* Cav. for the sake of convenience.

Buds of all stages of maturity were collected at various times of the day for the study of the developing embryo sac. For these early stages the young ovaries were dissected from the floral structures and embedded. Cross sections as well as longitudinal sections were made of these. The buds from each of the fruiting branches on a plant were collected and numbered with reference to their position on the plant; this was done to compare the relative state of development, and to secure closer stages in development.

For a study of fertilization, collection of ovules was begun at 8 A.M. of the day of flowering and continued at hourly intervals for forty-eight hours. The presence of mucilage material within the tissues of the ovary of cotton makes fixation of whole ovaries difficult. By far the best penetration of the fixative was secured where the ovules were dissected out and fixed separately. A sharp scalpel is used to remove the placentas with the attached ovules from the ovary; then the ovules which are obtained in rows of five or more are embedded. In this way all the ovules may be sectioned longitudinally at about the same angle and thus it is possible to secure median sections of embryo sacs.

The fixing agents giving most satisfactory results were Licent's and Bouin's. The material was dehydrated in alcohol and cleared in either xylol or chloroform. The latter seemed to cause less hardening of the tissues and was more satisfactory. Sections were cut from 7–15 microns in thickness and stained in a combination of safranin and gentian violet, or Heidenhain's iron-alum haematoxylin.

The Ovule

The ovules arise on the edges of the ingrown carpels as rounded masses of meristematic cells. The edge of the carpel becomes the placenta from which the ovules project; their funiculi appear rather long and massive at first in proportion to the size of the ovule primordia (Pl. LIV, fig. 2). The ovule curves backward and downward in its growth, producing a regular anatropous ovule. This curvature is completed by the time the megaspore mother cell is ready for its first meiotic division (fig. 4). The outer integument appears first, or about the time the inner integument appears, both growing over and around and practically covering the nucellus by the time of the first division of the megaspore mother cell. The outer integument grows somewhat faster than the inner integument and at the time tetrads are visible, surrounds the nucellus. The inner integument does not cover the nucellus at this stage. However this is quite variable in the different species. Figures 2, 4, and 9 show various stages of ovule development.

The inner integument of the ovule at the time of flowering consists of from ten to twelve layers of cells. The outer integument has a smaller number of layers of cells, usually from six to eight. One of these layers is of unusual importance and has for years been the subject of numerous investigations. This layer is the epidermis of the ovule, and it is important because it gives rise to the lint hairs. The differentiation of epidermal cells into lint hairs begins early on the morning of flowering; in fact elongation of some cells is noticeable at 8 A.M. Moreover in the early stages the initiation of lint development does not depend on pollination or fertilization because it begins about the time of the opening of the flower or shortly before.

Recent accounts of the early stages of this process have been contributed by Singh (8) and Farr (5) who report that the epidermis of the ovule continues to give rise to lint hairs for several days after flowering. It suffices here to say that Farr (5) includes a full review of the literature pertaining to lint development.

Comparison of Stages of Development

During the progress of this work it was found advisable to compare the stages of development of each bud on a plant. The fruiting habit of the genus *Gossypium* provides for the continuous formation of fruit branches and fruit buds throughout the cotton-growing season. Thus on a young fruiting branch are found the primordia of two or more flower buds which show various stages of development. As a result of this method of floral development the period of time required for flower primordia to develop into flowers is fairly constant.

Owing to various climatic factors the normal flowering sequence may be interfered with and, as often happens, the flowers do not open in the regular order of position as expected. Generally the difference in flowering dates between successive floral buds on the same fruiting branch is about six days

in Upland cotton. Approximately three days elapse between the opening of the first flower on one fruiting branch and the first flower on the next fruiting branch above.

This indicates that a similar relationship should exist in the development of the internal parts. All the buds from the plant were removed and labeled with the fruiting branch number and their position on the fruiting branch. When these buds were collected six fruiting branches were present on the plant. The results of these studies may be conveniently summarized as follows:

FRUITING BRANCH I

Bud 1. Taken day of bloom. Mature embryo sac; antipodals not present; beginning of lint.
Bud 2. Eight-celled embryo sac not completely organized.
Bud 3. Shows large megaspore mother cell.
Bud 4. Ovule primordia; beginning of integuments; archesporial cells present.
Bud 5. Carpel primordia present.

FRUITING BRANCH II

Bud 1. Eight-nucleate female gametophyte, partially organized; antipodals still present.
Bud 2. Two-nucleate female gametophyte, tetrad of megaspores, and in some ovules abortion of three megaspores.
Bud 3. Young megaspore mother cell present.
Bud 4. Ovule primordia present; fusion of carpel walls beginning.
Bud 5. Primordia of carpels present.

FRUITING BRANCH III

Bud 1. Eight-nucleate female gametophyte, not completely organized, polar nuclei migrating toward center.
Bud 2. Megaspore mother cell and tetrad of megaspores.

FRUITING BRANCH IV

Bud 1. Four-nucleate gametophyte.
Bud 2. Megaspore mother cell, young.

FRUITING BRANCH V

Bud 1. Megaspore mother cell.

FRUITING BRANCH VI

Bud 1. Young ovule showing sporogenous cells, origin of integuments.

As a result of these studies one can predict with reasonable certainty the stage of development of the female gametophyte or other organs of a bud collected next to an open flower, as well as the second, third, and fourth bud from this flower on the same fruiting branch.

THE ARCHESPORIUM

The development of the archesporial cell in cotton, in so far as has been observed, proceeds as in other dicotyledons. It has its origin from a subepidermal cell of the nucellus and arises before the differentiation of the

integuments. From its division a primary parietal cell and a primary sporogenous cell are formed (fig. 1). The integuments arise while the sporogenous cell is very young. While this sporogenous cell increases in size and becomes the megaspore mother cell, the primary parietal cell divides many times. The other cells of the nucellus also divide periclinally and anticlinally, thus embedding the megaspore mother cell in several layers of nucellar tissue. The mature megaspore mother cell is somewhat elongated and lies quite near the chalazal end (figs. 3 and 4).

Development of the Female Gametophyte

The megaspore mother cell which has been derived from the sporogenous cell now, by two meiotic divisions, produces a group of four megaspores. As is the general rule in angiosperms the three micropylar megaspores disintegrate very early, leaving the chalazal member of the tetrad to develop into the female gametophyte (fig. 5). Balls (1) reported the functioning of the micropylar megaspore, stating that the three chalazal members were the aborting ones; but this is quite uncommon and occurs in very few plants (3). This functioning of the chalazal megaspore was seen many times in all three groups of cotton studied, and there seems to be no evidence that any other member of the tetrad ever functions. From these observations, it is apparent that Balls mistook some slightly enlarged nucellar cells for aborting megaspores.

The functional chalazal megaspore now enlarges rapidly and may divide once before complete disintegration of the micropylar members (fig. 6). From this first division two nuclei result which may be termed the primary antipodal and the primary micropylar nuclei. These nuclei move apart and a large vacuole forms between them. The nuclei divide, forming two nuclei at each end of the sac. The two antipodal nuclei lie in the plane of the long axis of the embryo sac, while the micropylar members are transverse (fig. 7). The continued elongation of the embryo sac followed by increased vacuolation, proceeds on through the four-nucleate stage. When the eight-nucleate stage is reached, we find a rather long narrow gametophyte with a large vacuole and with the two groups of four nuclei at each end. The adjacent nucellar tissues undergo considerable disintegration during the development of the embryo sac. It has also been observed that the tissues of the nucellus and the integuments of the ovule remain meristematic for some time. The inner integument continues to increase in number of cells after fertilization, and during the development of the embryo builds up a mass of parenchyma several cells in thickness. The eight-nucleate structure, as in most dicotyledons, becomes organized into the mature female gametophyte (fig. 8).

Mature Female Gametophyte

As previously stated, the female gametophyte of all three species of cotton studied is ordinarily regular. The antipodals are present nearly one

week before flowering, but on the day of flowering all traces of antipodal nuclei have disappeared from the ovule. However, this is not unusual since ephemeral antipodals are found in a number of plants (3). The embryo sac lengthens somewhat and becomes slightly more vacuolate between the time of organization and the time of flowering. What follows is an account of the embryo sac that is found on the day of opening of the flower. Very little variation has been noted in the structure of the mature embryo sacs of the different species examined. The description given here is taken from Upland cotton of the Delfos variety and is typical of the others.

Sections made from ovules taken at nine o'clock of the day of opening of the flower show the membranes of the polar nuclei in contact, and the beginning of fusion is evident. The polar nuclei of some of the adjacent ovules in the same ovary are not in contact, but apparently migrating. An irregular or jagged mass of cytoplasm is present around the polar nuclei and continues as a thin layer down the side of the embryo sac wall and to the egg. Most of the embryo sac at this time consists of large vacuoles interspersed with threads of cytoplasm.

The synergids seem to be pyriform and show a filiform apparatus. The base of the synergid is rather pointed at the micropylar end (fig. 9). Each of the synergids contains a large vacuole in its end that is farthest removed from the micropyle. The nuclei of these structures lie near their middle and rather close to the edge of the micropylar end of the embryo sac. The synergid cytoplasm is very dense with the exception of the large vacuole. The form of the egg is slightly elongated or pear-shaped with the nucleus in its distal end. In size it is as large as or larger than one of the synergids. It is usually located between the synergids and lies free from the embryo sac wall except for a basal portion where it seems to be attached. The nucleus of the egg is embedded in a scanty mass of cytoplasm and is quite easily distinguished from the synergids and polars by the shape of the surrounding cytoplasm and by its location.

The general shape of the embryo sac is rather long and narrow, reaching to the chalaza on one end with its walls parallel, while there is a layer of from twenty to twenty-five cells of nucellar tissue at the micropylar end. The nucellus extends with about this same thickness on each side of the embryo sac. The nutrition of the developing gametophyte is supplied through the vascular bundle of the funiculus and from the distintegrating cells of the nucellus. There is also a large accumulation of starch at the chalazal end. This mass stains blue with IKI and red with safranin. The starch layer continues nearly around the ovule, just underneath the outer epidermis of the inner integument. This food reserve is used up during the development of the embryo. Just below this starch layer several thick walled cells are noted in the chalazal region. These stain red with safranin and appear to be lignified. Just how they function in the nutrition of the ovule is difficult to determine; it is probable that they have something to do

with the conduction of food to the embryo sac, since they join at its chalazal end and extend downward a short distance around the nucellus. There is no definite tapetal layer as in the anther, though many of the cells along the border of the gametophyte stain deeply and appear to be disintegrating. The form and orientation of the various parts of the embryo sac as found on the day of flowering are shown in figure 9.

In a few instances two completely organized embryo sacs have been found in ovules of Delfos cotton; these were fully developed and lying side by side in the nucellus. They were collected on the day of flowering. The cavity in the nucellus containing two embryo sacs was considerably larger than in ovules containing only one gametophyte. One ovule taken seventeen hours after opening of the flower contained two mature embryo sacs, the polars in one fusing, the polars of the other separate. The egg nuclei and synergids of both embryo sacs were normal. In these cases no doubt two megaspore mother cells were present and each developed, though in the enormous number of ovules of all varieties examined only one mother cell has ever been found in an ovule. It is possible that more than one megaspore of the tetrad functioned. So far as is known to the writer this condition has not been reported previously for any other Malvaceae. It is quite common in other plants (3). Certain features of the cotton ovule, especially the large amount of nucellar tissue and the massive integuments, resemble those of *Hibiscus trionum* described by Guignard (6). The late fusion of the polars is also found in many plants (3).

Fertilization

The number of hours between pollination and fertilization in cotton has been variously estimated as thirty-six to forty hours (1). This would of course vary somewhat under different temperature conditions as well as with the different species. Balls (1) found pollen tubes in the ovules and zygotes on the afternoon of the second day after pollination in Egyptian cotton.

Kearney (7) excised the styles of Pima cotton at eight hours, ten hours, and twelve hours, respectively, after pollination. Cutting off the styles eight hours after pollination failed to allow time for the growth of the pollen tubes and for the gametes to pass into the ovary; consequently no seed were set. But excision of the style ten and twelve hours after pollination did not prevent fertilization and the setting of a large number of seed.

According to the present investigations on Delfos material the time of the entrance of the pollen tube into the ovule is approximately fifteen hours after opening of the flower. This was not determined for Pima or Sea Island.

The styles of the three to five carpels are normally undiverged, but the stigmas are more or less distinct. The stigmatic surface is covered with a thick coating of long, somewhat pointed unicellular hairs. Microchemical

tests show the presence of many starch grains, and also show the tissues of the stigmas and styles to abound in starch at the time of flowering. Resin glands similar to those found in the stem, leaves, and other parts of the cotton plant are found in the tissues of the style. Well developed stomata are found in the epidermis of the style.

Many pollen tubes have been seen in the softer tissues between the vascular strands which traverse the style and stigma. The pollen tube appeared to be filled with a granular substance which stained with safranin, or when treated with IKI in chloral hydrate left no doubt of the presence of an abundance of starch. Inside the ovary pollen tubes were observed along the placenta and growing within the tissues of the placenta, indicating that the pollen tubes grow into the ovary through the placenta and on reaching the base of an ovule curve over and travel up the funiculus on the side next to the ovary wall where the micropyle of the ovule is directed. The placenta and funiculus are covered with a glandular layer which may secrete a nutrient fluid and thus facilitate the passage of the pollen tube to the micropyle.

The course of the pollen tube through the micropyle and the nucellus is interesting. As stated previously the micropyle of the ovule consists of two orifices passing between the two integuments. Where these two orifices do not coincide, as is usually the case, the pollen tube takes a zigzag course, sometimes even branching inwardly and going for a short distance between the two integuments. In other cases the openings coincide, but are at a slight angle to the long axis of the ovule. The opening through the outer integument is much the larger and somewhat flaring or funnel-form. The pollen tube in its travel through the nucellus to the embryo sac eats out or digests from two to four layers of nucellar tissue. The exact path is variable; sometimes after piercing the nucellus it follows in a straight line to the embryo sac, while in other cases the tube may take the easiest course, going for a distance between the inner integument and the nucellus (fig. 10). This gives rise to a slightly branched or haustorial appearance, which was evident in all the species and varieties under investigation. This confirms Balls' findings for Egyptian cotton (1). The extensive branching of pollen tubes reported by Guignard (6) for *Hibiscus* has not been seen in any of the cottons studied.

The receptive tissue described by Balls in the nucellus was not observed in the species investigated, though after the passage of the pollen tube through the nucellus two or more rows of disintegrating cells were evident. The tube enlarges into a bulb-like structure on reaching the interior of the embryo sac and presumably bursts, thus liberating the gametes. One synergid is usually destroyed by the entrance of the pollen tube, and in later material its remains can be seen as a deeply staining granular mass. Figure 10 shows the entrance of the pollen tube into the embryo sac and the position of the synergids and egg nucleus at this time. One synergid is usually de-

stroyed; the other synergid remains unharmed and persists for several days afterwards.

At the time of fertilization the position of the polar nuclei in reference to the egg varies slightly. In some cases the fusing polars surrounded by a mass of cytoplasm join the cytoplasm of the egg and synergids, while in other cases they are some distance away. The question whether there is a migration of the primary endosperm nucleus before the first division is somewhat doubtful and may be more related to the position of the polars at time of fusion than to any migration of nuclei. This variable position of polars has been seen both before and after fusion. There is some likelihood of distortion through fixative agents, which again must be considered in interpreting the position of these nuclei.

Since considerable variation is evidenced among the different ovules as to the time of fertilization, material collected twenty-six to thirty-two hours after opening of the flower provided excellent stages in fusion. Upland was at first studied more extensively, but several significant stages in Pima were also obtained.

The male nucleus, which is somewhat smaller than the egg nucleus, comes in contact with the nuclear membrane of the egg. Then the nuclear membrane disappears from between the two. At the time of fusion, as shown in Plate LV, figure 11, the somewhat thready chromatin of the nuclei appears to be in spireme, with distinct nucleoli. The male nucleus is slightly smaller than that of the egg. The appearance of the zygote shortly after fusion is shown in figure 12 with the two nucleoli still distinct. Considerably more cytoplasm is present around the zygote now than before fertilization, so it is easy to tell when fusion has been accomplished. The process of triple fusion is described later in connection with the development of the endosperm.

Development of Embryo

The following description of the early stages in embryo development has been taken from material of Mebane and Delfos. The exact time of the first division of the zygote was not determined for any of the species studied, though ovules from ovaries taken four to five days after flowering yielded young embryos in various stages. The first division of the zygote is transverse and gives rise to two cells of unequal size (fig. 20). The one nearest the micropyle may be designated as the suspensor cell. This cell, besides being larger, is more vacuolate and a trifle pointed. In a later stage in which the embryo contained eight to ten cells, three were quite definitely suspensor cells (fig. 21). In still older material a short massive suspensor consisting of two layers of cells is present (fig. 25). Balls (1) failed to find a suspensor in Egyptian cotton. His drawing of the octant stage of the embryo of Egyptian cotton was probably made from a section which was cut crosswise of the embryo and therefore would not show the suspensor. The embryo can be easily distinguished from the suspensor both in early and late

stages. The suspensor does not become very large and in older material is quite small in comparison with the embryo. The dermatogen of the embryo differentiates quite early, and in general the early stages in the embryo of cotton resemble those of typical dicotyledons.

Development of Endosperm

The way in which triple fusion is accomplished in Delfos shows some variability. The fusion of the polar nuclei has been seen in material taken a few hours after the opening of the flower and several hours before the entrance of the male gametes (fig. 26). In still later material this fusion is evidently complete, and when the sperms enter the embryo sac one unites with the egg and the other fuses with the already fused polar nuclei (fig. 27). Simultaneous fusion of all three nuclei has been seen occasionally in Delfos.

In most of the Pima material triple fusion proceeds as in Upland; that is, a fusion nucleus is formed before the inclusion of the male gamete. Sea Island was not observed as closely as Pima or Upland, but the stages obtained did not seem to differ from Upland.

In some Pima cotton grown in the greenhouse in the winter, an unusual condition was found which has not been observed in Upland or in Sea Island. One ovule collected at 8 P.M. of the second day of bloom shows what appears to be the simultaneous fusion of the male nucleus with the two polar nuclei (fig. 15). The two polar nuclei and the sperm nucleus are in contact, but the nuclear membranes of each are distinct. A common mass of cytoplasm surrounds all three nuclei, and the chromatin appears to be in the spireme condition. This is exactly what one would expect to find in triple fusion. However, in another ovule taken from the same locule as the one above, two such sets of triple nuclei were found. Since these two sets were rather close together and in the same mass of cytoplasm, it is evident that they are the result of the division of the three nuclei.

In another ovule from the same locule two similar groups of endosperm nuclei were found. In one of these groups all three nuclei were distinct and in the other group the wall had disappeared from between two of them (fig. 16). In still another ovule from this same locule a slightly later stage was found in which three distinct nucleoli were enclosed within the same nuclear membrane (fig. 17). The size of the nuclei described above is rather large. Possibly what happened was that the two polars and the sperm came together and the first division took place before the nuclear fusion was completed. From observations made on several sections of Pima cotton this seems to be the only possible interpretation. No delayed fusion of this kind was found in the Upland cotton. Endosperm has been found in ovules of Pima cotton which did not contain embryos. This might indicate in these cases that fertilization of the egg did not occur, or that if it did occur the embryo aborted. Development of endosperm without fertilization has been reported in several plants (3).

In reference to endosperm formation in Egyptian cotton Balls (1) states that "the triple nucleus resulting from the fusion of the second male and the two polar nuclei, moves up towards the egg, and on the evening of the day following pollination, two free ellipsoid nuclei are found, each with two large and one small nucleolus, the latter being certainly derived from the male nucleus." He states further that "these nucleoli retain their individuality for a few divisions." He describes the chromosome behavior as most irregular. So far as the writer has observed these divisions are quite regular in the many mitotic figures of endosperm nuclei observed in both Upland and Pima. Soon after fusion the primary nucleus divides. Many mitotic figures of this first division have been seen, and in Upland cotton this division is quite regular, as is shown in figure 13. This first division of the endosperm nucleus takes place quite close to the fertilized egg in some cases and farther back in the sac in others. The zygote at this stage shows two large nucleoli and somewhat thread-like chromatin in its nucleus. The cytoplasm surrounding this nucleus is somewhat the same as noted in other zygotes. After the first division of the endosperm nucleus no wall is formed between the resulting nuclei, neither is there any evidence of a rudimentary plate in this division. The two nuclei move apart some distance before dividing again.

Division proceeds rapidly, apparently simultaneously, and the newly formed nuclei form a single layer of endosperm which lines the embryo sac forty-eight hours after flowering. These free nuclear divisions continue until the embryo consists of from twenty to twenty-five cells, when wall formation in the endosperm occurs. This is brought about by furrowing in of the cytoplasm, thus cutting off one or more nuclei in each group (fig. 19). The endosperm fills the periphery of the embryo sac at this stage.

The massive endosperm which is developed in cotton is gradually absorbed by the growing embryo until at maturity there remains only a thin layer, one or two cells in thickness, surrounding the much-folded cotyledons.

Summary

1. Studies on the development of the female gametophyte, and early stages of embryo development, are reported for Sea Island, Pima, and Upland cottons.

2. The embryo sac develops from the chalazal megaspore of the tetrad; the complete structure is very long and embedded in many layers of nucellus.

3. The antipodals degenerate early. The polars begin fusing before entrance of the male gametes to form a polar fusion nucleus.

4. The pollen tube enters the ovule fifteen to twenty hours after pollination and by digesting its way through the nucellus reaches the embryo sac. Slight branching of the tube in the region of the integuments was noted.

5. Fertilization is completed from twenty-four to thirty hours after opening of the flower.

6. The endosperm in cotton usually results from the fusion of the second male gamete with the polar fusion nucleus. Various methods of triple fusion are reported.

7. A massive endosperm is developed only to be resorbed by the growing embryo.

8. The embryo develops a short suspensor.

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EXPLANATION OF PLATES

The following figures were drawn with the aid of an Abbé camera-lucida under apochromatic objectives, compensating oculars, and aplanatic condenser. The approximate magnifications are given for each figure.

PLATE LIV

FIG. 1. Primordia of ovule with sporogenous cell. Pima. × 600.

FIG. 2. Longitudinal section of young ovule showing young integuments and sporogenous cell. Figure 1 was taken from this section. Pima. × 125.

FIG. 3. Megaspore mother cell just before reduction division. Sea Island. × 600.

FIG. 4. Young ovule from which figure 3 was taken. Sea Island. × 125.

FIG. 5. Tetrad of megaspores. The three micropylar ones disintegrating. Sea Island. × 600.

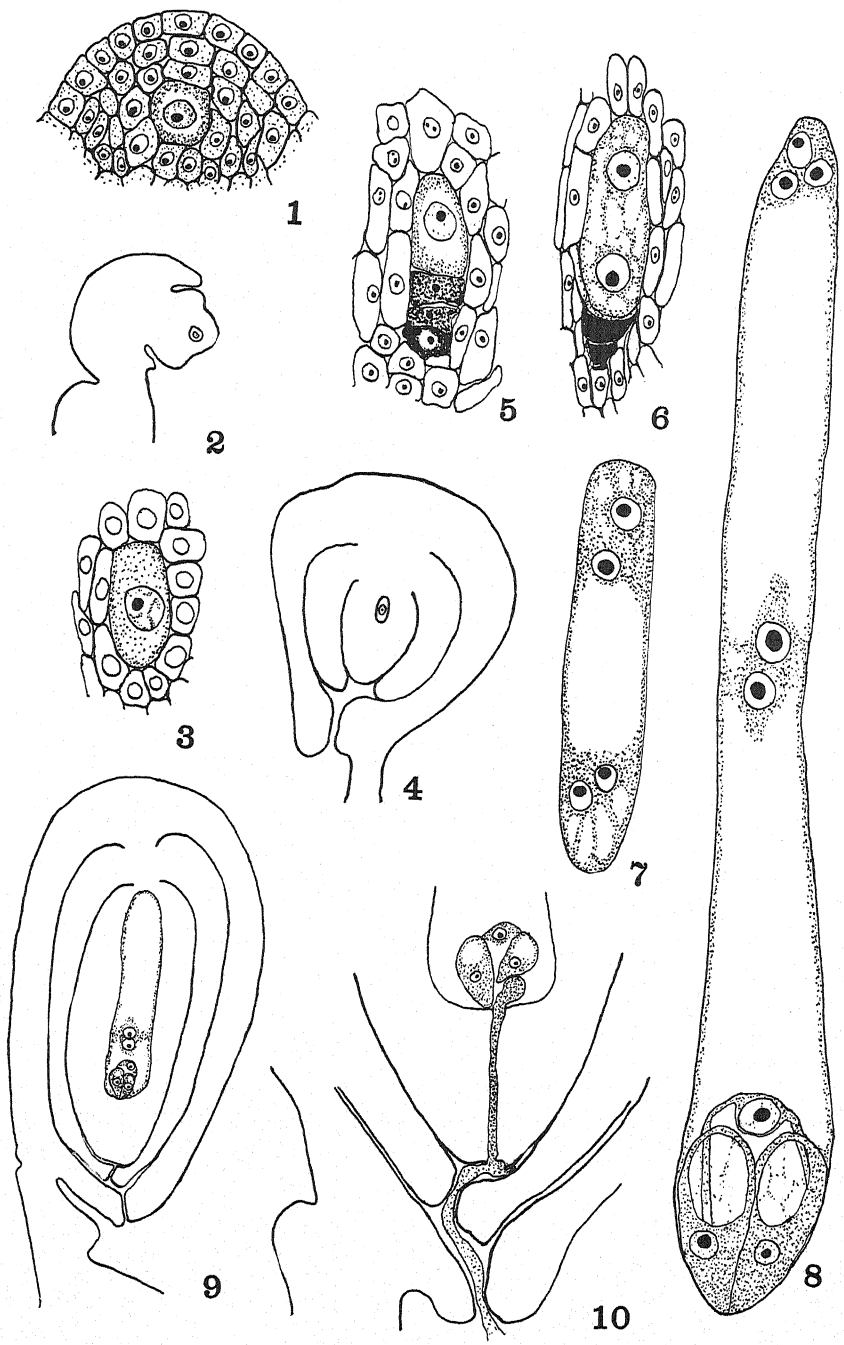
FIG. 6. Two-nucleate female gametophyte. Three aborting megaspores. Upland. × 600.

FIG. 7. Four-nucleate female gametophyte. Upland. × 600.

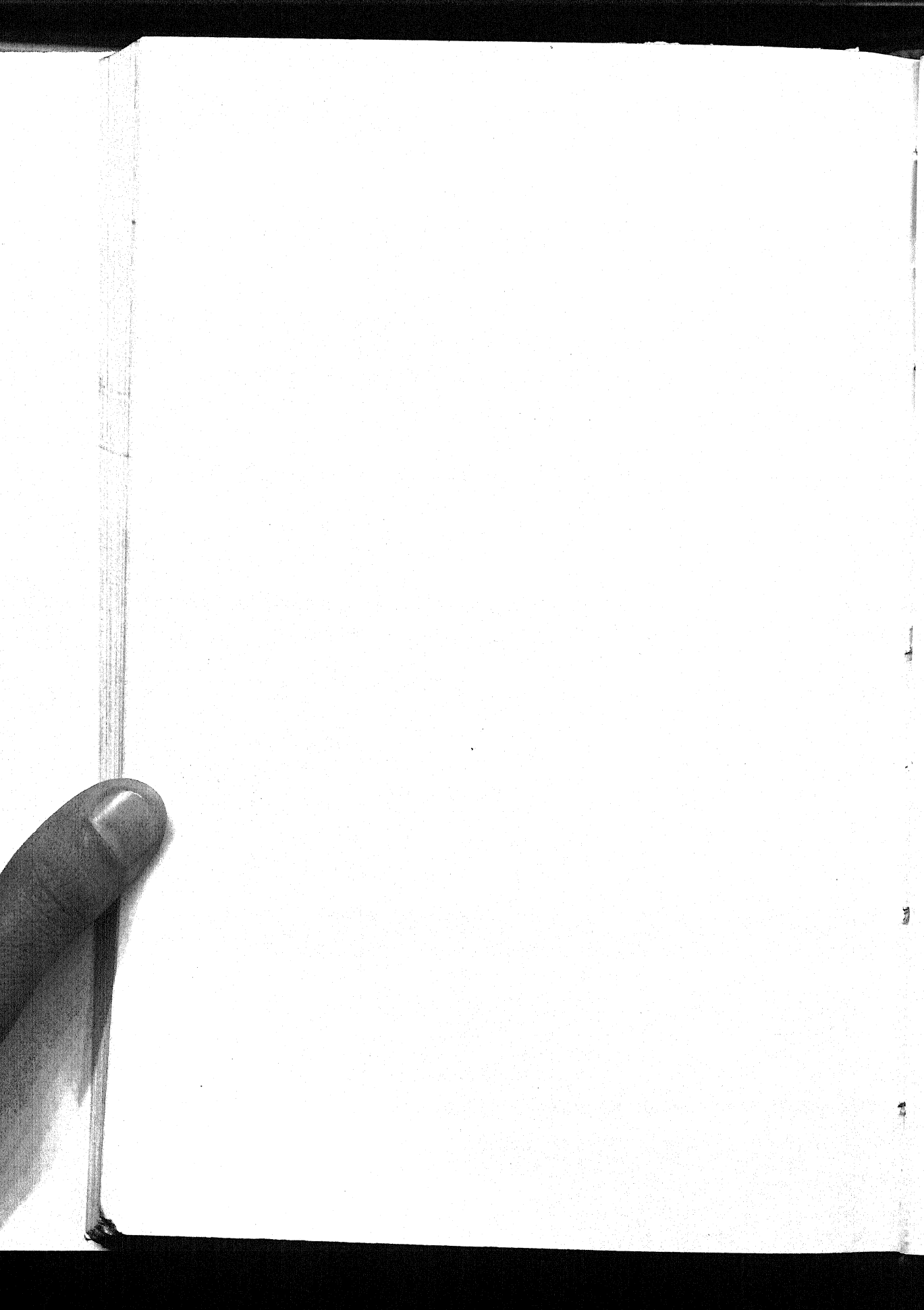
FIG. 8. Completely organized female gametophyte showing the two synergids, egg nucleus, polar nuclei, and the antipodals approximately one week before flowering. Pima. × 600.

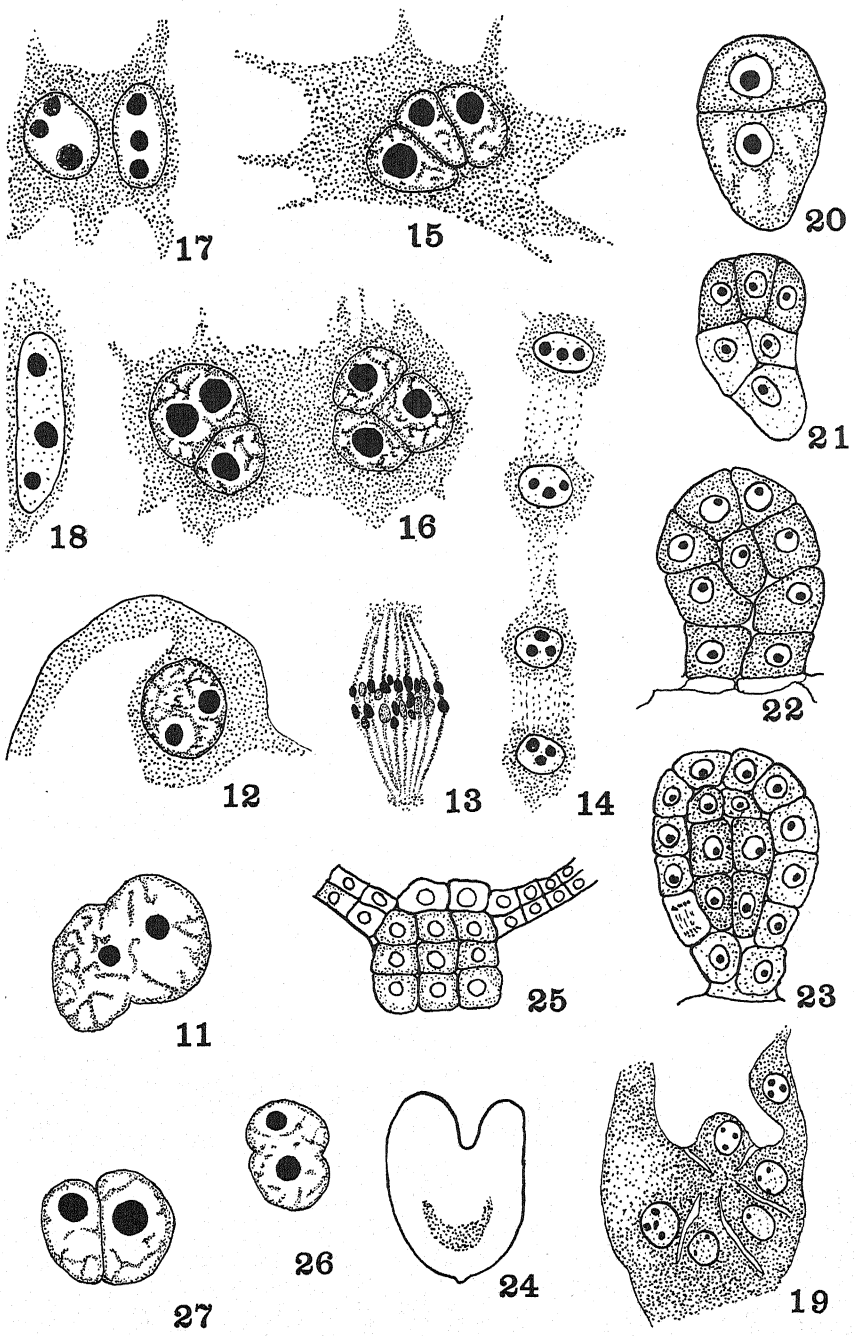
FIG. 9. Longitudinal section of ovule taken on the day of flowering. Upland. × 60.

FIG. 10. Portion of ovule showing growth of pollen tube through integuments and nucellus. Pima. × 125.



GORE: GAMETOPHYTE OF COTTON





GORE: GAMETOPHYTE OF COTTON

PLATE LV

FIG. 11. Union of sperm with egg. Pima. × 1100.

FIG. 12. Zygote showing chromatin material of egg and sperm thirty-three hours after flowering. Upland. × 1100.

FIG. 13. Metaphase of first division of primary endosperm nucleus. Upland. × 1100.

FIG. 14. Four endosperm nuclei in ovule from which figure 12 was taken. Upland. × 600.

FIG. 15. Triple fusion. The nuclear membranes distinct. Pima. × 600.

FIG. 16. Two groups of nuclei which are formed by the division of the polars and sperm before fusion. Fusion is beginning in one of these groups. Pima. × 600.

FIG. 17. Endosperm nuclei. Pima. × 600.

FIG. 18. Elongated endosperm nucleus found in Upland. × 600.

FIG. 19. Wall formation in endosperm by furrowing. Delfos. × 600.

FIG. 20. Two-celled embryo. Delfos. × 600.

FIG. 21. Eight- to ten-celled embryo, suspensor lightly shaded. Delfos. × 600.

FIG. 22. Embryo of twenty or more cells. Mebane. × 600.

FIG. 23. A later stage showing differentiation of dermatogen. Mebane. × 600.

FIG. 24. Young embryo showing differentiation of cotyledons. Mebane. × 125.

FIG. 25. Suspensor and differentiation of root cap. Mebane. × 600.

FIG. 26. Fusion of the two polar nuclei. Delfos. × 600.

FIG. 27. Union of sperm with polar fusion nucleus. Upland. × 900.

SCLEROTINIA WILT OF GREENHOUSE SNAPDRAGONS[1]

J. J. TAUBENHAUS AND W. N. EZEKIEL

(Received for publication March 27, 1932)

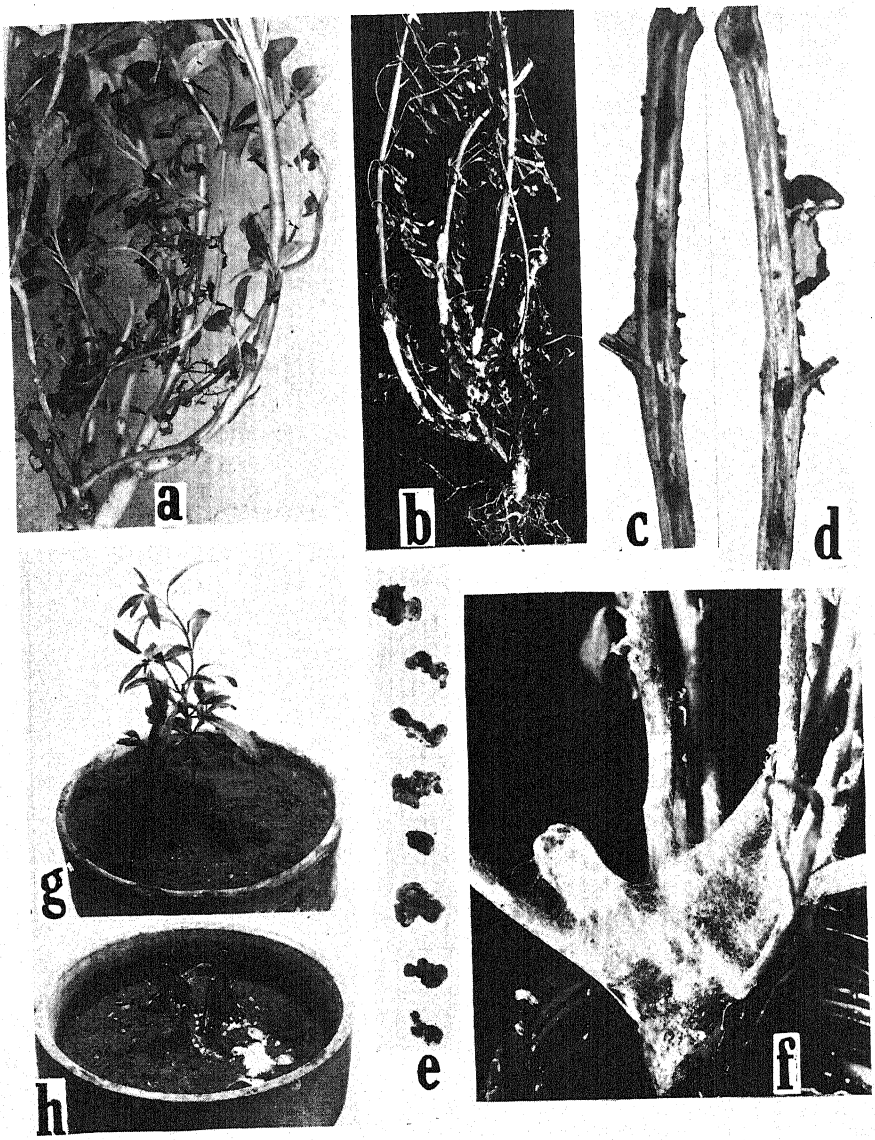
Our attention was called in October, 1927, to a serious disease of snapdragons grown in a commercial greenhouse at Temple, Texas. About 60 percent of the plants had been killed, causing a loss of about $20,000. The owner believed that the trouble was brought about by the prolonged cold, cloudy, and rainy weather then prevailing, which interfered with the proper ventilation of the greenhouse.

The disease was thoroughly distributed in the three beds of the house. Plants were found in all stages of infection. Early infections appeared as water-soaked lesions overrun by thin wefts of white fungus growth. Individual branches, particularly those closest to the ground, soon wilted (text fig. 1, *a*). The shrunken stems were covered with a copious white wefty mycelial growth with numerous large sclerotia (fig. 1, *b* and *f*). These occurred on the outside and inside of the affected roots and stems of the plants (fig. 1, *c, d, e*). As large portions of the stems and branches became involved, entire plants wilted and fell to the ground, often within 24 to 48 hours after infection, forming mats of dead and wilting plant material which were overrun by the dense growth of white mycelium and sclerotia. The fungus apparently spread by direct contact of the collapsed, infected plants with adjoining normal plants, as well as on the surface of the soil. In an area of 64 square feet of an infested snapdragon bed, 466 sclerotia were collected. Of these, 171 were on the surface of infected stems; 92 were in the inside of these stems; and 203 were formed on the ground.

The appearance of infected plants and the numbers of sclerotia found suggested a *Sclerotinia* as the probable cause of the trouble. Petri dish isolations from infected tissues and from the sclerotia yielded a growth resembling *S. sclerotiorum*. Healthy snapdragon plants were then inoculated with this culture and also with *S. sclerotiorum* previously isolated, respectively, from apothecia produced under infected fig trees,[2] from celery, and from beans. Snapdragon seedlings were planted in 8-inch pots in February, in a cool greenhouse held at about 70° to 75° F. After two months, these plants had attained considerable size and were used for inoculations. Pure cultures of the *Sclerotinia* strains mentioned were grown on sterilized bean pods, which inoculum was placed on the ground next to the uninjured stems, and the plants

[1] Published with the approval of the Director as Contribution No. 208. Technical Series, of the Texas Agricultural Experiment Station.

[2] **Taubenhaus, J. J., and W. N. Ezekiel.** A Sclerotinia limb blight of figs. Phytopathol. 21: 1195–1197. 1931.



TEXT FIG. 1. Sclerotinia wilt of greenhouse snapdragons; *a*, early natural infection, white mycelial growth on one erect stem and another stem already collapsed; *b*, final stage, entire plant killed and covered with mycelium of the fungus; *c* and *d*, sclerotia of the fungus inside diseased stems; *e*, individual sclerotia; *f*, profuse mycelial growth developed on infected stems in moist chambers; *g*, check, uninoculated plant, and *h*, one of plants inoculated with *S. sclerotiorum* isolated from infected snapdragons.

then covered with bell jars for 48 hours. As summarized in table 1, the inoculated snapdragon plants developed a wilt similar to that observed in the greenhouse irrespective of the various strains of *Sclerotinia* used as inoculum (text fig. 1, *g* and *h*). The fungus was readily recovered in all cases.

TABLE 1. *Inoculation of Normal Snapdragon Plants with Cultures of Sclerotinia sclerotiorum from Various Hosts*

Source of Cultures Used as Inoculum	Number of Plants Inoculated	Number of Plants Killed
Snapdragon	35	29
Apothecia under diseased fig trees	10	7
Celery	20	16
Bean	16	12

Young and Morris [3] in their studies of *Sclerotinia* wilt of sunflowers caused by *S. libertiana* include the snapdragon in their list of 49 hosts. It may be noted that the writers have not found *Sclerotinia* to attack any plants grown in the open in the calcareous Houston black clay soils typical of Central Texas and predominating in Bell County, in which Temple is located; although *Sclerotinia* commonly attacks lettuce, beets, carrots, and anise [4] in South Texas, and fig trees along the Gulf Coast. It is thus difficult to account for the sudden appearance of the disease on greenhouse snapdragons in Central Texas, unless it was introduced with infested manure or other refuse used in the compost. This seemed a plausible explanation since snapdragons grown that year in other greenhouses in the vicinity of Temple were free from the disease.

In December, 1927, a number of sclerotia from infected snapdragon plants were collected in the Temple greenhouse and brought into the laboratory at College Station. After air-drying for twelve weeks, the sclerotia were buried about one-half inch deep in moist Houston black clay soil in 24 pint Mason jars, using twelve sclerotia per jar. The jars were left uncovered at room temperature, and were watered regularly without being flooded. After fourteen weeks, apothecia developed from eight sclerotia in one of the Mason jars, and from ten sclerotia in another. The apothecia, asci, and ascospores agreed in size and shape with *S. sclerotiorum*. The sclerotia in the other containers from which no apothecia developed were found to be decayed and overrun by a *Trichoderma*. There appears no doubt that the fungus which attacks the greenhouse snapdragon plants can be safely referred to *Sclerotinia sclerotiorum* (Lib.) Mass.

[3] **Young, P. A., and H. E. Morris.** Sclerotinia wilt of sunflowers. Montana Agr. Expt. Sta. Bull. 208: 3–32. 1927.

[4] **Taubenhaus, J. J., W. J. Bach, and W. N. Ezekiel.** Anise drop. Plant Disease Reporter (Mimeographed), Bur. Pl. Ind. 13: 141. 1929.

Summary

A serious wilt disease was found on fall greenhouse snapdragons in the vicinity of Temple, Texas. The disease was artificially reproduced by inoculation with *Sclerotinia* isolated from the snapdragon and from other hosts, and the fungus reisolated. The sclerotia produced apothecia, asci, and ascospores typical of *Sclerotinia sclerotiorum* (Lib.) Mass.

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THE CHROMOSOMES OF *LYCOPERSICUM PIMPINELLIFOLIUM* [1]

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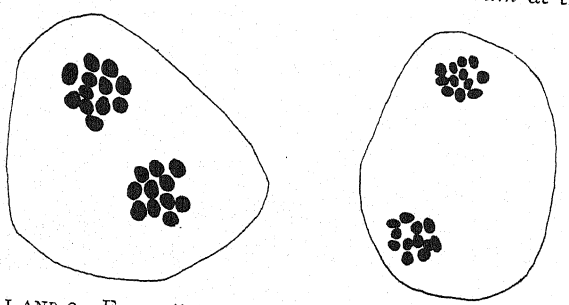
(Received for publication March 29, 1932)

The chromosome number of the common tomato, *Lycopersicum esculentum* Mill., has been reported under the name *Solanum lycopersicum* as being 12 pairs. The red currant tomato, *Lycopersicum pimpinellifolium* Dunal. is recognized by most taxonomists as a separate species. It differs from *L. esculentum* in having no long foliage hairs, the pubescence being restricted to the scarcely visible gland hairs; the plant is weaker, with a slenderer, more spreading, more finely branched growth habit, and with distinctly smaller and thinner leaves. The fruit is globular, two-loculed, and about 10–12 mm. in diameter. The flowers are also somewhat smaller than those of *L. esculentum*. The chromosome number of this species has not been reported, and it is the purpose of this note to record the results of a study of the chromosomes of *L. pimpinellifolium*, and to make some cytological comparisons between this species and *L. esculentum*.

The material from which the drawings were made was taken from greenhouse cultures of genetically pedigreed stocks, and killed in Allen-Bouin's fixing solution, imbedded in paraffin, and stained with iron-alum-hematoxylin. The drawings were made with a Zeiss binocular microscope equipped with a 100 × fluorite objective and 25 × compensating oculars. A camera lucida was used. The magnification of the figures is × 2125.

Text figure 1 shows the chromosomes of *L. esculentum* at the metaphase



TEXT FIGS. 1 AND 2. FIG. 1 (left). Metaphase II of *Lycopersicum esculentum*. FIG. 2 (right). Metaphase II of *Lycopersicum pimpinellifolium*.

of the second or homotypic division. This stage was entirely satisfactory for making counts, and for comparing chromosome sizes. Figure 2 is a

[1] Paper No. 48, Department of Genetics, Iowa State College, Ames, Iowa.

pollen mother cell of *L. pimpinellifolium* at the same stage. There are twelve chromosomes in each half of the cell, this being the haploid number for the species. A glance at the figures will show a distinct difference in size between the chromosomes of the two species. The chromosomes of *L. esculentum* had an average diameter of 1.12 microns. Those of *L. pimpinellifolium* had an average diameter of 0.77 of a micron. The chromosomes are not quite spherical but slightly ovate. For this reason two measurements were made on each chromosome and the total of 100 measurements on 50 chromosomes used in securing the average in each case. The sizes were so constant within each species that more measurements seemed unnecessary.

Comparative measurements were made on the pollen mother cells in various stages as well as on mature pollen grains. Except in the case of the pollen the sizes were so nearly alike that the slight differences which did occur were of no significance. Furthermore, the cells varied so much in shape in early stages that a true average diameter is difficult to obtain. However, the measurements of the pollen gave rather striking results. The pollen of *L. pimpinellifolium* has an average diameter of 21.6 microns and an average volume of 5277 cubic microns when immersed in 45 percent acetic acid. That of *L. esculentum* has an average diameter of 25.01 microns and an average volume of 8191 cubic microns. The size of the pollen grains within the species was relatively constant. In *L. pimpinellifolium* the range was from 20.0 to 23.0 with but five out of 100 reaching 23 microns. In *L. esculentum* the range was from 23.5 to 27.0 microns. There was but one of each of these extreme diameters in 100 grains.

It is interesting to note that while the chromosome number is the same for the two species, there are significant morphological differences in the chromosomes and pollen grains. Furthermore, the microscopical morphological comparisons parallel the gross morphological characters. The tendency in each case is toward smallness and fineness in *L. pimpinellifolium* and toward an increase in size and coarseness in *L. esculentum.*

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THE ANATOMY AND DEVELOPMENT OF THE FLORAL ORGANS OF *BUGINVILLAEA GLABRA*

D. C. Cooper

(Received for publication April 6, 1932)

This investigation of the anatomy and development of the floral organs of *Buginvillaea glabra* Choisy was carried on in connection with a study of microsporogenesis (Cooper, 1929, 1931). The floral development of members of the Nyctaginaceae has thus far been more or less neglected. Fischer (1880) briefly mentioned the development of the macrospore mother cell of *Oxybaphus nyctagineus*. According to Heimerl (1889), Finger (1887) described the anatomy and the development of the flower of *Mirabilis Jalapa*. Dahlgren (1916) and Woodcock (1928) have since made studies of the development of the ovule in this species, and the latter author (1929) also reported on the development of the ovule of *Thelygonum Cynocrambe*, a member of the Cynocrambaceae, a family showing close relationship to the Nyctaginaceae. Rocén (1927) made a comparative study of pollen and embryo sac development in eight species belonging to the Nyctaginaceae, *Buginvillaea glabra* being one of the species studied. The most extensive report on any one member of this family is that of Maheshwari (1929), who has described the development of the floral organs of *Boerhaavia diffusa*.

A description of the materials used in this study and the details of fixing and staining have already been outlined (Cooper, 1931). Some useful preparations to show the emergence of the floral organs were made as follows: very young buds were fixed in formal-acetic-alcohol, stained in light green, and mounted *in toto* in glycerine. When it seems necessary, comparisons will be made with material of the horticultural variety of *Buginvillaea*, Crimson Lake, which is considered to be a variety of *B. spectabilis* Willd.

Observations

The small and inconspicuous flower of *B. glabra* is borne upon the upper surface of a brightly-colored lavender bract, its pedicel being attached to the thick mid-vein near the base of the bract. The flowers seldom stand alone; usually they are three together in an umbel-like structure. The bracts, which are somewhat heart-shaped, are so arranged that their edges touch, thus forming a corolla-like structure out of which three inconspicuous tubular flowers project (text fig. 1*a*).

Inflorescences each bearing from three to five clusters of bracts are borne in the axils of the leaves. The perfect cream-colored, tubular flower is from 6 to 7 mm. in diameter and from 14 to 18 mm. in length. The perianth is

roughly pentagonal and terminates in five equal lobes. Lateral outgrowths of the perianth lobes give the open flower a somewhat frilled appearance (text fig. 1*b*).

The androecium consists of seven to eight (usually eight) stamens with long flattened capillary filaments (Pl. LVI, fig. 10). Usually two or three stamens extend beyond the perianth tube (text fig. 1*b*). The single pistil bears a long hairy stigma and a relatively short style (fig. 10). The mature ovary is about two millimeters in length. The ovule has a form somewhat intermediate between the anatropous and the campylotropous types.

The floral parts develop in acropetal succession: perianth, stamens, carpel. A careful study has been made to determine if primordia of another whorl

TEXT FIG. 1. *a*, an inflorescence of *Buginvillaea glabra*. × 2.
b, an open flower. × 2.

of floral structures might have started development and then ceased, but no evidence for such a whorl could be obtained. Maheshwari (1929) similarly found no evidence for a fourth whorl in *Boerhaavia diffusa*.

The inflorescence is first recognized as a small rounded mass of cells in the axil of a leaf. As this young peduncle elongates, three bract primordia arise from the edge of its somewhat flattened apex. When the terminal inflorescence has reached some size, two lateral inflorescences may develop from its peduncle.

The individual flowers appear as club-shaped masses of meristematic cells in the axils of the partially developed bracts (fig. 2). As the bract grows, each pedicel, which remains attached adnate to the mid-vein of the adjacent bract, elongates so that when the flower is mature it appears to have arisen from the upper surface of the bract.

The first primordium of a perianth lobe to appear is on that side of the meristematic protuberance away from the bract (fig. 3). The other four

perianth primordia appear in quick succession and in their growth gradually broaden at their bases (fig. 4) until they unite to form a ring of meristematic tissue. The growth of this ring produces the perianth tube. Division of the cells within the primordia causes an upward extension of the perianth lobes. These lobes roof over the apex of the pedicel (fig. 5).

The primordia of the perianth lobes are laterally as well as terminally meristematic (figs. 7*a*, *b*, *c*). Consequently these lobes widen above the tube and their edges fold inward (figs. 8*a*, *b*, *c*, 9). When the bud unfolds, there are wing-like appendages on either side of each perianth lobe (text fig. 1*b*).

The primordia of the stamens emerge in a single cycle (fig. 5). The single primordium of the carpel arises at one side of the growing tip, and as it develops, its base broadens laterally until it gradually encircles the apex of the growing pedicel. The apical portion of the pedicel becomes the terminal nucellus. The primordium of the carpel, by continued growth, encloses this nucellus.

The young stamen is early differentiated into a filament and a terminal anther (fig. 10). The anther is at first a mass of similar cells surrounded by an epidermis, which is more or less circular in cross section. With continued growth the anther, in cross section, becomes oval, and then rectangular (fig. 11). Shortly thereafter four lobes appear and simultaneously one or occasionally two rows of hypodermal cells at each corner of the anther become somewhat more prominent than the rest of the cells. Their large nuclei and their somewhat greater affinity for the stain make them conspicuous. One or two such archesporial cells appear in cross section in each anther lobe (figs. 11, 12). The archesporial cells become divided periclinally into primary sporogenous and primary parietal cells (fig. 13). The primary parietal cells divide periclinally and anticlinally until four cell layers of parietal tissue are formed (fig. 14). The outermost parietal layer later becomes differentiated into an endothecium and the innermost layer into the tapetum. Division of its sporogenous cells occurs, until ultimately there are eight to ten spore mother cells in each lobe as seen in cross section (fig. 15).

The microspore mother cells are at first polyhedral, but with continued enlargement of the anther they become somewhat spherical and their nuclei undergo the reduction divisions. At about the time when the spore mother cell nuclei reach the stage of synizesis, the nuclei of the innermost layer of parietal cells divide mitotically (fig. 16). The cells of both the endothecium and the tapetum increase greatly in size, whereas the cells of the intermediate wall layers show little growth, but become considerably flattened. As the tapetal cells continue to develop, the two nuclei of each remain in a centrally located mass of dense cytoplasm (fig. 17). In anthers in which the nuclei of the microspore mother cells have reached the heterotypic anaphases (Pl. LVII, fig. 18), the two nuclei of each tapetal cell are seen to be closely adjacent to each other. They may ultimately become flattened against each other and occasionally an appearance is produced as of a stage in amitosis. No tapetal cells were found, however, with more than two nuclei.

Tischler (1908) states that mitosis occurs in the young tapetal cells of the hybrid *Mirabilis Jalapa* × *M. tubiflora*. The two nuclei of a tapetal cell come into contact, being flattened on their adjacent surfaces. In the older tapetal cells Tischler finds several nuclei of varying sizes. Rocen (1927) figures mitosis in the young tapetal cells of both *Buginvillaea glabra* and *Oxybaphus micranthus*. In older tapetal cells of *Buginvillaea glabra* he finds that the two nuclei persist, whereas in corresponding cells of *Oxybaphus micranthus* there are numerous nuclei. An examination by the writer of the tapetal cells of *Buginvillaea spectabilis* has revealed a situation similar to that found in *B. glabra*. In sections of buds of *Oxybaphus nyctagineus,* mitotic figures were found in the cells of the young tapetum and not more than two nuclei could be found in older tapetal cells. Maheshwari (1929) reports that the tapetum of *Boerhaavia diffusa* is constantly binucleate.

After the completion of the reduction divisions, the microspores become thick-walled and lie loosely in the pollen sacs (fig. 19). The tapetum now disorganizes and the wall layers between the two adjacent microsporangia on one side of the anther become stretched, the cells break apart, and a single theca is formed. Simultaneously with the disorganization of the tapetum, the cells of the endothecium, with the exception of a few cells at the stomium or region of dehiscence, develop the characteristic thickened spiral bands on the cell walls which are concerned with dehiscence. The cells of the epidermis of the anther with the exception of those in the region of the stomium have at this stage become thick-walled.

Shortly after the flower opens, the anthers dehisce. The pollen grains which are shed are in reality microspores. The thick exine and intine of these spores seems to aid in the retention of their approximately hemispherical shape. As earlier reported (Cooper, 1931), the content of the microspore is shrunken and no two-celled pollen grains were observed. At no time has the germination of a pollen grain been noted.

The single cauline ovule develops within the closed carpel (fig. 20). An apical hypodermal cell becomes differentiated (fig. 21 *a, b*) as a primary archesporial cell while the nucellus is still upright and at about the time when the carpel actually closes over the apex of the pedicel. The primary archesporial cell differs from its neighbors in being larger and in having a larger nucleus. This cell divides to form a primary parietal cell and the macrospore mother cell. The primary parietal cell divides (fig. 22) several times so that the macrospore mother cell is deeply imbedded in the nucellus (fig. 23).

Shortly after the macrospore mother cell is formed, the integuments arise at a level just below this cell from the epidermal layer of the nucellus (fig. 22). The outer integument is the first to appear, and soon after its appearance and just above it the primordium of the inner integument is developed. The entire ovule grows more rapidly on that side away from the point where the primordium of the carpel made its appearance, so that it bends toward that direction (fig. 23). The bending of the ovule takes place in the region of the

origin of the integuments (fig. 24), and continues until the ovule assumes a form intermediate between the campylotropous and the anatropous conditions (figs. 25, 26). Woodcock (1928, 1929) describes a single campylotropous ovule as occurring in both *Mirabilis Jalapa* and *Thelygonum Cynocrambe*. Maheshwari (1929) reports that there is a single basal anatropous ovule in *Boerhaavia diffusa*. The outer integument of *Buginvillaea glabra*, which becomes several layers of cells in thickness, and the inner integument, which remains two layers of cells in thickness except at the apex in older stages, grow so that they have reached a level even with that of the end of the nucellus by the time the macrospore mother cell nucleus has reached the stage of diakinesis (fig. 24). No regular difference in size between the cells of the two layers which make up the inner integument similar to that described for *Mirabilis Jalapa* (Woodcock, 1928) was noted. When the flower opens the integuments have grown so as to form a short micropyle (fig. 26). That portion of the inner integument which surrounds the micropyle becomes four or five layers of cells in thickness.

The presence of two integuments does not appear to be a constant characteristic of the Nyctaginaceae. An examination of preparations of *Buginvillaea spectabilis* revealed the presence of two integuments in that species. Rocen (1927) found two integuments in *B. glabra, Oxybaphus nyctagineus,* and *O. viscosus,* and only one integument in *Mirabilis Jalapa,* whereas Maheshwari (1929) described only one integument for *Boerhaavia diffusa*.

As already described (Cooper, 1931), the macrospores may disintegrate early so that a nucellus containing vegetative tissue only is developed, or a macrogametophyte may be formed. In many cases the ovule has begun to disintegrate before the opening of the flower. There is an almost total sterility in the female line.

There are many multicellular glandular hairs on both sides of the perianth primordia as well as on the bracts, immature leaves, and young stems. These hairs arise from epidermal cells each of which divides to form a primary hair cell and an epidermal cell (fig. 29). Figure 29 shows an anaphase stage of nuclear division in a primary hair cell. By successive divisions of the basal cells of the hair (fig. 30) a row of from seven to fifteen cells is formed. The cells of the immature hairs are unequal in size (fig. 32), but in older hairs the cells are similar in size and shape. The cells of the hairs found on the young stems and leaves possess plastids, whereas there are no plastids in the cells of the hairs borne on the bracts and perianth tube. The apical cells of these hairs become very much enlarged, somewhat flattened at the apex, and develop thick walls (fig. 31). The cytoplasm shrinks from the cell wall and becomes so very dense that it is difficult to distinguish the nucleus (fig. 33). In the older apical cells a secondary layer of material is laid down within the cell wall (fig. 34). The apex of the terminal cell appears to contain a very small pore. These hairs are quite different from the short-stalked glandular hairs with spherical terminal cells found by Maheshwari (1929) on the surface of

the perianth of *Boerhaavia diffusa*. According to Solereder (1909), the enlarged terminal cells of the glandular hairs found in the Nyctaginaceae are in most cases ellipsoidal or clavate, more rarely spherical.

The stalk of the mature ovule is thickly covered with cylindrical, single-celled papillate hairs (fig. 28). Rocen suggests that the surfaces of these hairs offer a suitable support for the growing pollen tube.

Raphides are found in the spongy tissue of the young bracts, in the parenchymatous tissue of the stalk and walls of the ovary, and in the style (fig. 20). They begin development in the vacuole of an enlarging cell (fig. 35) in a manner similar to that described by Smith (1923) for the raphides of an orchid. By continued growth they rupture the wall of the parent cell and so come to lie between the cells of the surrounding tissue. When they have reached their full development, a bundle of needle-like crystals is formed which is several times as large as the cell in which it originated (fig. 26). The presence of raphides is common in certain tissues of the members of the Nyctaginaceae. Solereder (1908) notes their presence in species of *Buginvillaea, Cryptocarpus, Mirabilis, Oxybaphus, Neea, Phaeoptilum,* and *Pisonia.*

In an early stage of development of the umbel-like inflorescence, a definite abscission layer is differentiated in the peduncle, a short distance below the attachment of the bracts. This region is easily recognized, when observing the matured inflorescence, as a slight swelling of the peduncle about one half inch below the bracts. An examination of a longitudinal section of this region shows that the cells of the abscission layer have remained small, rounded, and thick-walled, whereas those both above and below along the axis of the peduncle have grown greatly, appearing rectangular in section, and have remained thin-walled (fig. 27).

Perhaps two or three days after the opening and withering of the flowers of the cluster, the peduncle breaks at the level of the abscission layer and the entire inflorescence drops away. Examination of numerous such dropped flowers from plants growing in the greenhouse at the University of Wisconsin failed to reveal the presence of either fruit or seeds. Examination of several hundred inflorescences that had been collected from beneath plants which were growing in the open in both Florida and Brazil likewise failed to show the presence of either fruits or seeds.

Summary

1. The flower cluster of *Buginvillaea glabra* consists of three flowers having an umbel-like arangement. A single cream-colored flower is attached adnately to the upper surface of each lavender-colored bract.

2. An inflorescence bearing from three to five clusters of flowers is borne in the axil of a leaf.

3. The mature flower consists of a perianth tube which terminates in five equal lobes, seven or eight (usually eight) stamens, and a single carpel.

4. The young flower appears as a protuberance in the axil of a bract. The order of appearance of the floral organs is perianth lobes, stamens, carpel.

5. The development of lateral extensions of the perianth lobes above the tube produces wing-like extensions on either side of the lobe.

6. The stamen consists of a long, flattened, capillary filament and a small terminal anther.

7. Microsporogenesis is typical and four microspores are produced.

8. When the microspore mother cells are in an early prophase stage (synizesis), the nuclei of the innermost parietal layer divide mitotically; the tapetal cells are thus binucleate.

9. The upright cauline ovule bends upon itself so as to take a form that is somewhat intermediate between the campylotropous and anatropous types.

10. The surfaces of the bracts and perianth lobes are covered with numerous multicellular glandular hairs.

11. Raphides are found in the spongy tissue of the bracts, perianth lobes, and ovary wall.

12. An abscission layer is formed in the peduncle, just below the level of attachment of the bracts.

13. The umbel-like inflorescences drop away shortly after the withering of the flowers.

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EXPLANATION OF PLATES

All drawings were made with an Abbé camera lucida at table level. Spencer compensating oculars, a Leitz 16 mm. objective, a Spencer 8 mm. N.A. 0.60 apochromatic objective, and a Leitz achromatic oil-immersion N.A. 1.32 objective were used. The magnification was as indicated for each figure. All figures have been reduced approximately three-fifths in reproduction.

PLATE LVI

FIG. 2. A young inflorescence with a bract (*B*) removed to show the floral primordia (*F*). × 200.

FIG. 3. A young flower in the axil of a bract. Two whorls of primordia can be seen. (*B*) bract, (*P*) perianth lobe, (*S*) stamen. × 100.

FIG. 4. A somewhat older flower. × 100.

FIG. 5. A still older flower with two perianth lobes and a portion of the bract removed. The apical portions of the stamens (*S*) are beginning to enlarge. The carpel (*C*) has almost completely closed over the nucellus. × 100.

FIG. 6. Diagrammatic cross section of a flower, showing the relative position of the floral organs. (*B*) bract, (*P*) perianth, (*S*) stamen, (*C*) carpel, (*O*) ovule. × 100.

FIG. 7. (*a*) A transverse section through the perianth lobes of a young flower, showing the lateral outgrowths of the lobes. (*b*) A section through the same perianth at the level at which the lobes are uniting to form the perianth tube. (*c*) Section through perianth at a lower level. × 100.

FIG. 8. (*a*) A transverse section through the perianth lobes of a somewhat older flower. (*b*) A section through the same perianth at the level at which the lobes are uniting to form the tube. (*c*) Section at lower level. × 100.

FIG. 9. A reconstructed drawing of a series of longitudinal sections of a flower, to show the infolding of the perianth lobes. × 100.

FIG. 10. The mature staminal whorl and ovary. Note the hairy stigma of the pistil. 8 stamens are present. × 15.

FIG. 11. A transverse section of a young anther, the archesporial cells shaded. × 700.

FIG. 12. An archesporial cell and surrounding cells. × 1550.

FIG. 13. An archesporial cell has divided into a primary parietal and a primary sporogenous cell. × 1550.

FIG. 14. Portion of a longitudinal section through an anther showing division figures in both parietal and sporogenous cells. × 1550.

FIG. 15. A transverse section through a portion of an anther, showing the development of the wall layers, and division of the sporogenous cells to form microspore mother cells. × 800.

FIG. 16. A section through a portion of an anther showing the differentiation of an endothecium and division figures in the tapetal cells. × 800.

FIG. 17. A section through a portion of the anther at a somewhat later stage. × 1000.

PLATE LVII

FIG. 18. A still later stage. × 1000.

FIG. 19. A section through a portion of an anther showing the disintegrating tapetum (*t*), an endothecium (*e*) with the characteristic markings, the thin walled cells in the region of the stomium (*s*), and the break (*b*) developing between the two sporangia. × 700.

FIG. 20. A longitudinal section through a young pistil, showing position of raphides in ovary wall. × 200.

FIG. 21. (*a*) Longitudinal section of young ovule showing archesporial cell. × 800. (*b*) Archesporial cell. × 2900.

FIG. 22. Longitudinal section through an ovule. The macrospore mother cell is much larger than the surrounding cells. The primary parietal cell has divided anticlinally. The outer integument is beginning to develop. × 800.

FIG. 23. A somewhat older ovule. The macrospore mother cell is more deeply imbedded in the nucellus. The integuments and nucellus are developing more rapidly on one side, so that the ovule is bending. × 800.

FIGS. 24, 25, 26. A series of diagrams showing the bending of the ovule as it develops. × 200.

FIG. 27. A portion of a longitudinal section through the abscission layer of the peduncle. × 800.

FIG. 28. A few papillate hairs from the stalk of the ovule. × 800.

FIG. 29. Very early stages in the development of the glandular hairs. × 1550.

FIG. 30. A portion of a glandular hair showing a division figure in one of the basal cells. × 800.

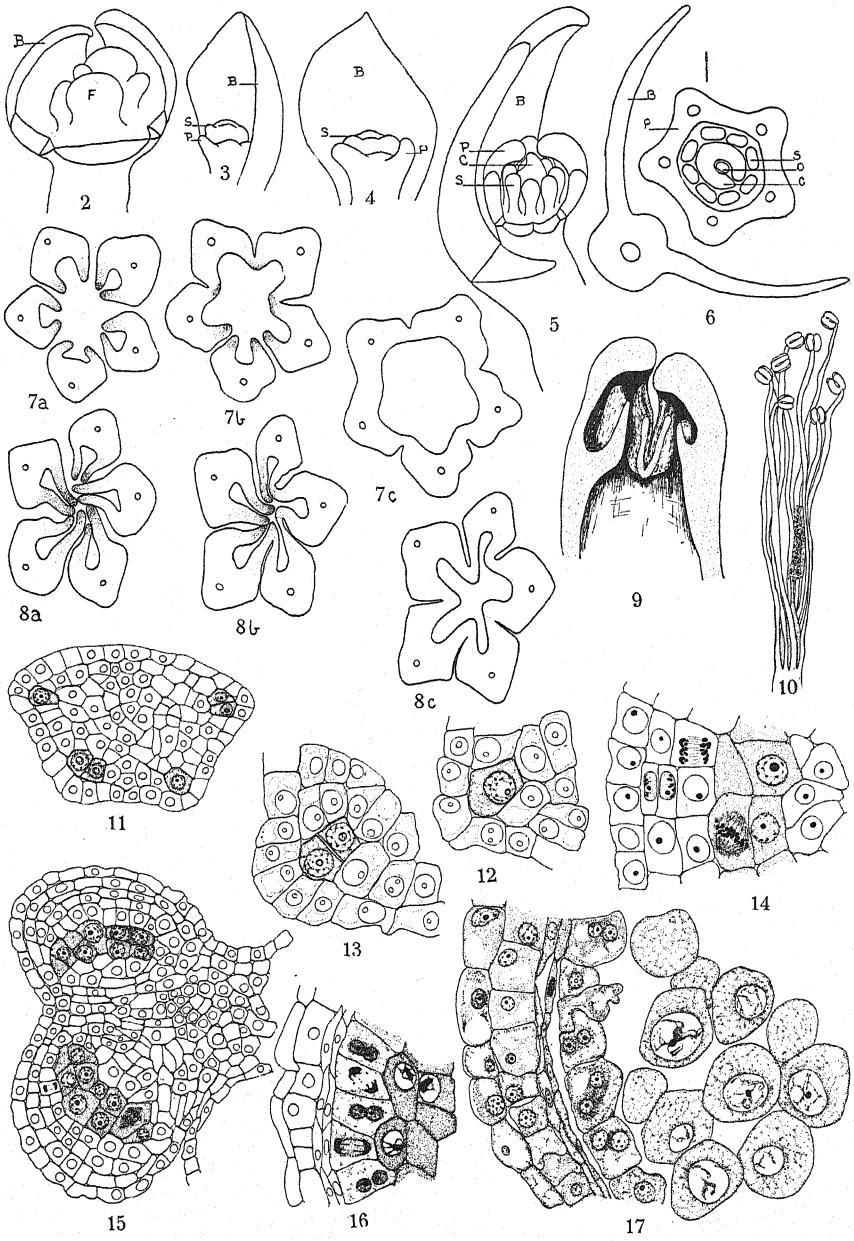
FIG. 31. Terminal cell of young glandular hair. × 1550.

FIG. 32. A young glandular hair from a young leaf. Plastids are present in the cells. × 800.

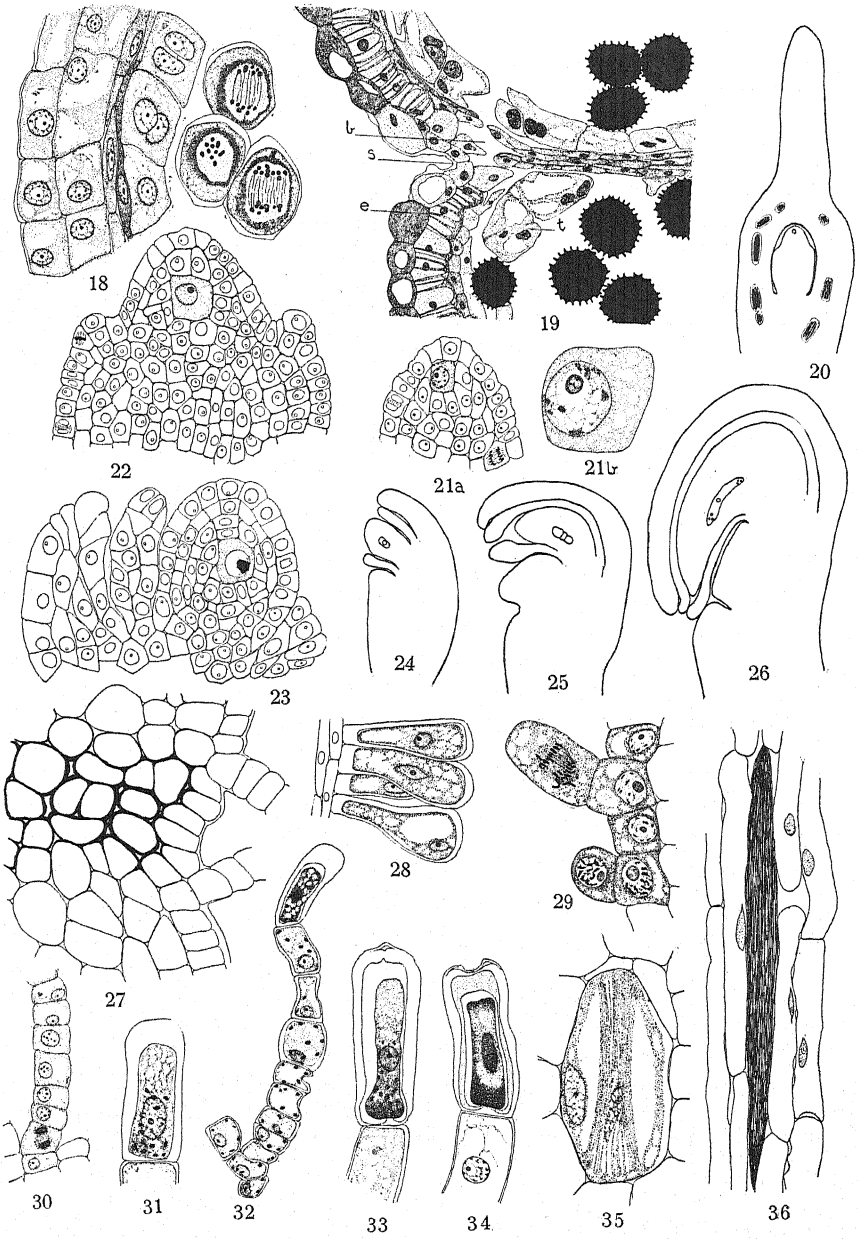
FIGS. 33, 34. Older stages in the development of the terminal cell. The cytoplasm shrinks from the thick cell wall. In figure 34 a substance, evidently secreted by the cytoplasm, is being laid down against the cell wall. × 1550.

FIG. 35. A cell from the wall of a very young ovary. A bundle of raphides is beginning to develop in the central vacuole of the cell. × 1550.

FIG. 36. A fully developed bundle of raphides, between the cells of the style. × 800.



COOPER: BUGINVILLAEA



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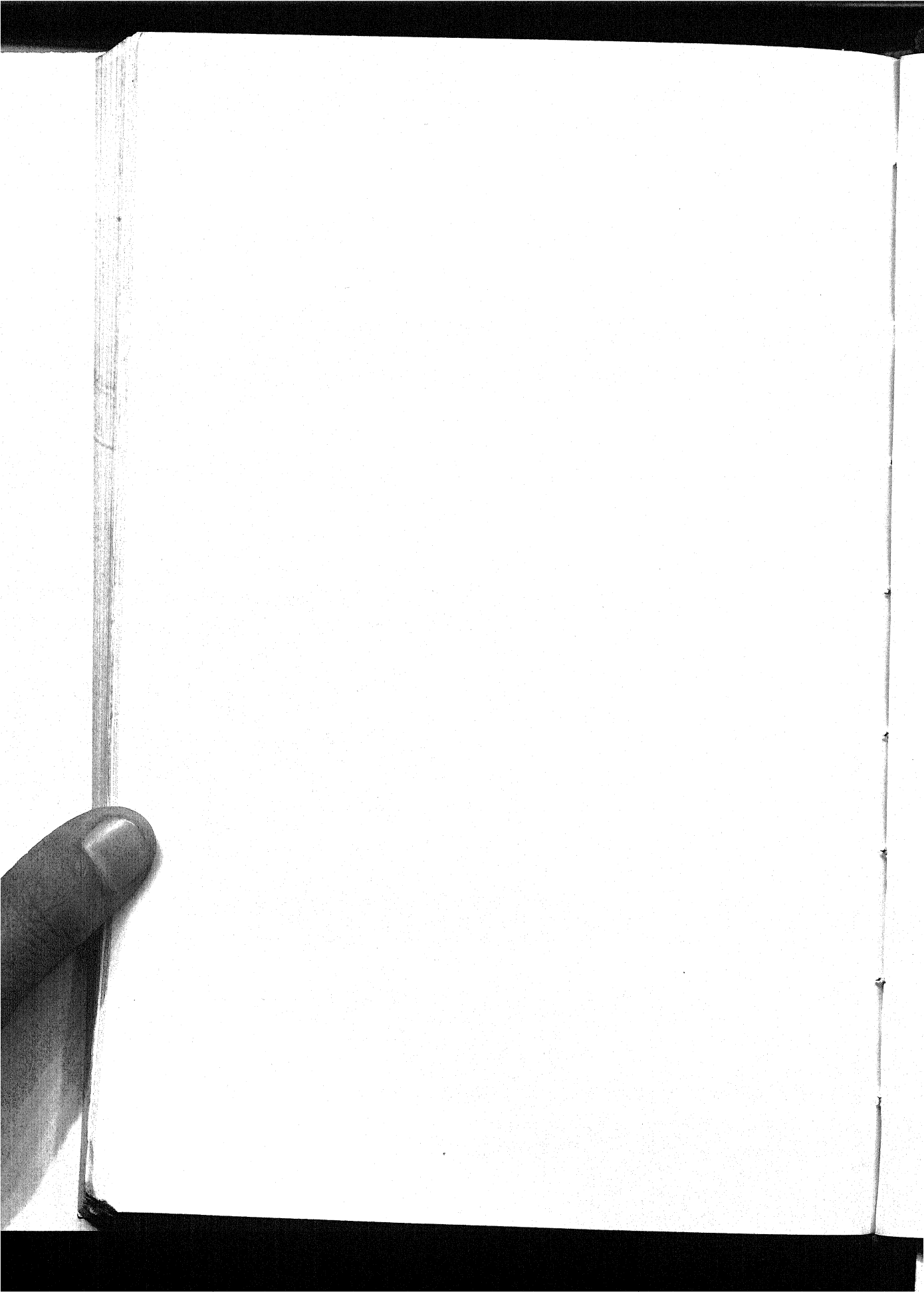


ANAEROBIC RESPIRATION OF CACTI[1]

FELIX G. GUSTAFSON

(Received for publication April 9, 1932)

The writer's studies of anaerobic respiration of several different varieties of fruits and such underground parts as tubers and rhizomes have now been extended to the fleshy structures of cacti. This work was done at the Desert Laboratory of the Carnegie Institution of Washington at Tucson, Arizona, during the winter months of October to January. Although the climate in Tucson is rather mild as winters go, yet it was by no means warm. There were several periods when for one night or several nights in succession the temperature was below freezing. For the greater part of the time there was not much growth of any kind and certainly not in any of the cacti used in these experiments.

The temperature of the bath in which the experiments were conducted gradually rose during a period of three and a half months from 25° to 25.5° C., but during an individual experiment, lasting usually a week, the change was so slight that it could not be detected. For respiration chambers large-mouthed pickle jars were used. Otherwise the experiments were conducted as in the past (1). In order to establish an equilibrium, the plants (kept in the dark) were in the respiration chambers, through which air was drawn, usually for about 20 hours before the collection of CO_2 was commenced.

Six species of cacti and the leaves from two species of thin-leaved plants were used. The leaves were used to get a comparison between fleshy plants and thin-leaved structures from the same habitat and under identical conditions. The plants used were: *Carnegiea gigantea, Ferocactus wislizeni, Neomammillaria microcarpa, Echinocereus fendleri, Opuntia engelmannii, O. versicolor,* and the leaves of *Verbena ciliata* and *Encelia farinosa.* The cacti ranged in compactness from the nearly spherical *Ferocactus* to the flat *Opuntia engelmannii* and the long and cylindrical growth of *Opuntia versicolor.* (The names as here used are those given by Britton and Rose, "The Cactaceae.")

To eliminate the activity of bacteria or fungi all plants used were first washed free from soil and other foreign matter and were always healthy and uninjured, except where joints were broken off or roots cut off, and in one experiment with *Ferocactus wislizeni* in which a fungus infection developed. Sometimes these injured surfaces healed before the plants were introduced into the respiration chambers, or they were coated with vaselin or grafting wax, although in some experiments joints with fresh cuts were placed in

[1] Paper from the Department of Botany of the University of Michigan, No. 377.

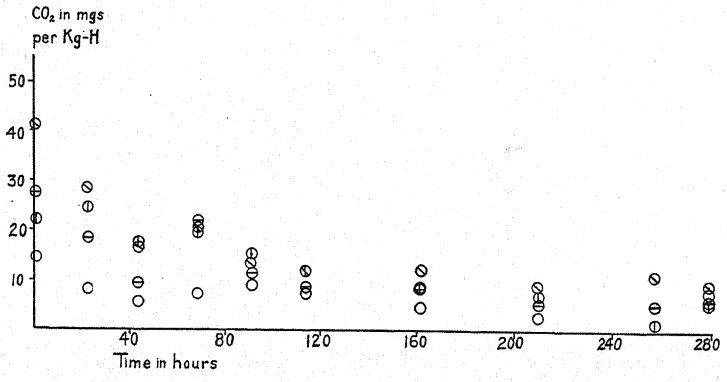
the chambers. When the plants were taken out of the chambers at the end of the experiments they were always examined for fungus or bacterial action, but only in the instance mentioned above were evidences of infection noted in the plants that had been in nitrogen. In a few instances the plants in air had fine wefts of fungus on the roots. It seems to the writer that bacterial and fungal responsibility for the CO_2 production under the experimental conditions can very definitely be excluded.

CARNEGIEA GIGANTEA

More experiments were conducted with this plant than with any other, partly because it is typical of this region and partly because small specimens were easily available. Dr. Shreve had on hand a large number of *Carnegiea gigantea* plants, grown from seeds, that were seven years old and these were very satisfactory for the work. Larger plants were easily obtained from the mountains. In all, seven experiments with controls were conducted with the giant cactus or " Sahuaro," as it is more commonly called in Arizona.

To get some idea of the normal respiration of cacti, over an extended period of time, an introductory experiment was conducted in which two different sizes of *Carnegiea gigantea* were used. Each group was run in duplicate. The plants grown in the greenhouse were used and they ranged in size from 3 to 4 inches in height. The larger plants (about 6 inches) were collected in the field.

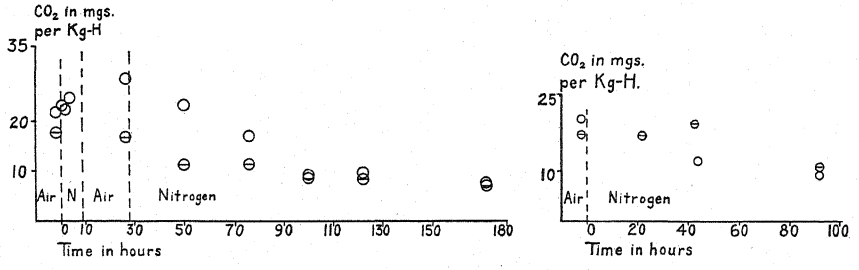
From this introductory experiment (text fig. 1) it will be seen that the



TEXT FIG. 1. Respiration in air of plants of *Carnegiea gigantea* of two different sizes. The plain circles and the circles with the horizontal line through them represent two experiments with 7-year-old plants 3 to 4 inches in height. Three plants were used in each experiment. The other two experiments were conducted with older plants, 6 inches in height. Each circle represents the average of two collections. This is true for all of the experiments cited in this paper. The plants represented by the plain circles and the circles with the perpendicular line through them were taken out of the chamber 231 hours after the first collection was made, or 275 hours after the plants were put in the dark chambers, and placed in the sun out of doors for 7 hours. They were returned to the chamber and collections made next day as before.

respiration of cacti is very low when compared with other plants. In only one collection was the amount of CO_2 produced more than 30 mgs. per kilogram hour. This is low even when compared with ripe fruits. Collections were made in this experiment for a period of 280 hours, and the plants were in darkness for about 320 hours, yet the respiration was fairly well sustained. In fact there was only a slight decrease after the first hundred hours. To find out whether organic substances were running low the plants from one container from each group were taken out after having been in darkness for 275 hours and placed in the sun for 7 hours. They were then returned to the containers and measurements made the following day as before. The small plants showed an increase for the two days that followed, while the large plant did not. In fact its CO_2 production was less the day after it had been in the sun than it had been before.

In experiment 8 (text fig. 2) 3 seven-year-old plants were used. They



TEXT FIGS. 2 AND 3. FIG. 2 (left). Respiration of 7-year-old *Carnegiea gigantea* plants. Three plants were used in each respiration chamber. The plain circles represent the plants in nitrogen in all experiments except those in text figure 1. FIG. 3 (right). Respiration of *Carnegiea gigantea* plants, 8 inches tall.

varied in size from 2½ to 4½ inches in height. They had been in nitrogen only 9½ hours when air was again introduced because of a leak in the valve supplying the nitrogen from the tank. The next day nitrogen was again introduced and the plants remained in nitrogen continuously for 142 hours. The plants in nitrogen maintained a higher rate of CO_2 production for two days than did the plants in air. After that the rate was practically the same for control and experimental plants. When the plants were taken out of the respiration chamber the smallest plant in the nitrogen showed a slight browning on several ribs near the apex. The other two seemed to be in good condition. After some days the plants were put in soil and kept in the greenhouse. The two smaller plants developed blackening at the apex after some time and were discarded. The third plant was still in good condition two and a half months after the experiment was ended. The blackening noticed may not have been due to injury after all, as other plants from the same lot growing in the greenhouse have also developed black apices, without having been in nitrogen.

In experiment 42, which was conducted in January, *Carnegiea gigantea* was also used. These plants were 8 inches tall and 4 inches in diameter. The respiration in these plants was very low (text fig. 3); the highest only 20 milligrams per kilogram per hour. It is to be noted that the plants in this experiment had been exposed to low temperature for several weeks before they were used. This may to some extent account for the small amount of CO_2 produced in comparison with the other two experiments cited above, which were conducted in October, when the temperature was much higher. In this experiment there was no great difference in the rate of the control and the experimental plant, except for one day. The plant which had been in nitrogen showed a patch of yellow-gray color on one side near the apex. On cutting into the plant it was found that the chlorophyll had partly disintegrated, becoming yellow-brown (instead of the usual bright green). The chlorophyll disintegration extended into the plant for only 2 or 3 millimeters.

Two attempts were made to use plants over a foot tall, but the containers could not be maintained air tight for any length of time. One plant was in an atmosphere of nitrogen for nearly two days. During this time the CO_2 production was actually much higher than it had been while in air.

All experiments with *Carnegiea gigantea* show that this plant is capable of living in an atmosphere free from oxygen for several days with only superficial injuries and some plants were not at all injured. During this time they maintained a rate of respiration which did not differ very much from the control. In some it was a little lower while in others a little higher than the control.

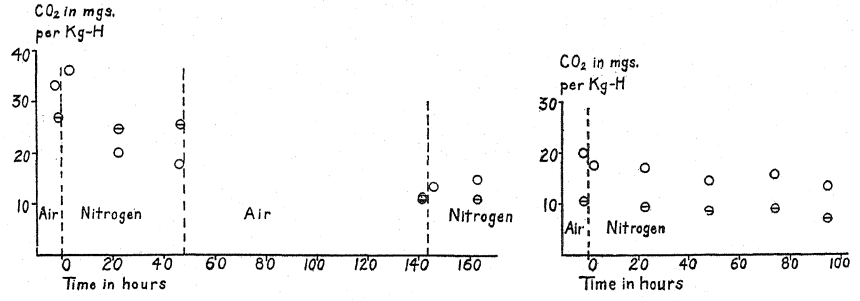
Ferocactus wislizeni

This plant is an interesting subject for experiments in anaerobic respiration. In outline it is almost a perfect sphere with ridges running perpendicularly. This supplies a relatively small surface for a large volume and therefore the gas exchange might also be expected to be slow, with high content of CO_2 and low content of oxygen, at least in the central part of the plant. If the interior is normally low in oxygen content, these plants would be expected to suffer less injury and respire more, when in total absence of oxygen, than plants which normally have high oxygen content in their interior.

Four experiments, each one with a control, were run with this plant. The rate of respiration, either aerobic or anaerobic, was very low; in two of the experiments the CO_2 production was less than 20 mgs. per Kg-H. The others were all less than 40 mgs. per Kg-H. except in one reading, where it was 42.

In experiment 10 and its control the plants weighed 381.8 and 326.9 g., respectively, and measured about 4 inches in diameter, not including spines. As shown by text figure 4 the plant in nitrogen respired more than the control at the beginning of the experiment. The next day, 20–24 hours

after nitrogen introduction, the experimental plant respired only 60 percent as much as it did in air the preceding day, and the control had decreased only a few percent. Next day there was only a slight decrease. At the end of 48 hours the nitrogen was replaced by air, and the plant remained in it for 94 hours. At the end of the aerobic period two collections were made from each one of the plant chambers. It was found that the two plants respired approximately the same at this time. The air was replaced by nitrogen and the next day the plant in nitrogen showed an increase over the plant in air. When the plants were examined at the end of the experiment.



TEXT FIGS. 4 AND 5. FIG. 4 (left). Respiration of *Ferocactus wislizeni* plants, about 4 inches in diameter. FIG. 5 (right). Respiration of *Ferocactus wislizeni* plants, 4 inches in diameter. Note the very low rate of respiration.

eight days after they were placed in the containers in the dark, they were both found to be in perfect condition. They were planted in soil and placed in the green house. Two months later when again examined they were found to be in perfect condition. No new roots had been formed in either one, but that was not to be expected at this time of the year.

The other experiments showed no appreciable decrease in the respiration at the end of 24 hours in nitrogen. In experiment 15 (text fig. 5) there was a slight decrease immediately after placing the plants in nitrogen, but after that the decrease in CO_2 production was approximately the same as that in the control plant. The control plant respired considerably less than the plant in nitrogen throughout the experiment. These plants were of approximately the same size as the plants in experiment 9. At the end of the experiment both plants were in perfect condition and when examined six weeks later they were found to be healthy and uninjured in every respect.

One of the two remaining experiments was conducted with small plants and the other with plants of the same size as in the cited experiments. In one of these the respiratory rate increased somewhat after the first day in nitrogen and this higher rate was maintained throughout the experiment. At the time this behavior was very puzzling but it later developed that the plants were infected with a fungus. This experiment should therefore be discarded in summarizing the results with *Ferocactus wislizeni.* In the

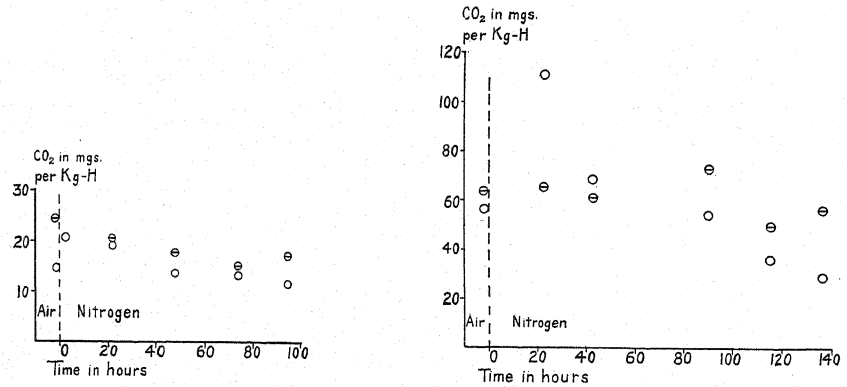
fourth experiment, in which small plants were used, the rate gradually fell, but nearly the same in the control and the experimental plants. From these four experiments it seems certain that *Ferocactus wislizeni* is capable of carrying on anaerobic respiration for a considerable time with no injury to the plants.

Neomammillaria microcarpa

This plant is rather small, usually about one and a half to two inches in diameter and from two to four inches tall, with small tubercles all over the surface. This plant therefore has a much larger surface per unit volume than does *Ferocactus wislizeni.*

Three experiments with controls were performed with this species. In all experiments several plants were used in each respiration chamber, because the individual plants were so small.

Experiment 13 with its control is typical and it is here illustrated by text figure 6. Four plants slightly different in size were used in each jar. Similar plants were selected for the two chambers. All plants were collected on the same hillside, a few days before they were used in the experiment. This



Text Figs. 6 and 7. Fig. 6 (left). Respiration of *Neomammillaria microcarpa* plants. Four plants were used in each respiration chamber. Fig. 7 (right). Respiration of *Opuntia versicolor* joints. About 15 joints were used in each jar. Most of the joints were quite plump and short.

procedure was followed in all the experiments with *Neomammillaria microcarpa.*

It will be noticed that the plants in air respired considerably more at the beginning than the plants which were put into nitrogen in spite of the careful selection of similar plants.

During the first 24 hours the plants in nitrogen respired more than they had done when in air, but after that the rate fell below the original, though not as much as in the control. At the completion of the experiment the plants appeared normal except that the chlorophyll was of a brownish-green

cast, and after some weeks the chlorophyll had disappeared entirely. To the touch they felt a little softer than the control plants. Microscopic examination of thin sections showed no definite difference between the control and the experimental plants. Six weeks after the plants were removed from the respiration chamber the three plants saved were again examined. They had been planted in sand in the meantime. One plant was dead. The other two plants showed a very interesting development. The tubercles which had shown the disintegration of chlorophyll were in many instances ready to slough off the outer layer and underneath this white and dry layer the tissue was of a bright green color showing active growth and development of new chlorenchyma tissue.

The other two experiments were in the main like the one described in detail. One of them showed a greater drop in nitrogen than the experiment cited. The plants were saved in only one experiment and after two months one very small one was dead, while the other four were alive and the outer injured layer of the tubercles was sloughing off and new chlorenchyma tissue had developed underneath.

On the whole *Neomammillaria microcarpa* showed a greater decrease in respiration when oxygen was excluded than did *Ferocactus wislizeni*. It was also injured superficially and perhaps internally to a greater extent. Only the smallest plants, however, were injured beyond the surface layer, *i.e.*, the chlorophyll-bearing layer, extending for two to three mm. below the surface.

Opuntia versicolor

Opuntia versicolor and *O. engelmannii* were both used to make complete the survey of the more common cacti found around Tucson.

Opuntia versicolor which has previously been used in several investigations on respiration is a profusely branched form, with cylindrical joints. The end joints are three-quarters of an inch to an inch in diameter. Joints separate very easily from one another and do not have to be cut apart, as a slight wrenching will separate them. Terminal joints were used in the two experiments conducted with this plant. Whether the terminal joints used were last year's growth or older cannot be stated, as not all apices will produce new joints every year. Material used in both experiments was obtained from the same large plant. Opposite or otherwise similarly situated joints were broken off and each one put in a separate lot. In this way the experimental joints and the control joints were as similar as was possible to get them.

In one of these experiments there was a very large increase in respiration immediately after the nitrogen was introduced. This increase lasted at least more than 24 hours. According to Richards (2) the CO_2 production decreased to about half a few hours after the joints were put in nitrogen, when the temperature was maintained at 21° C., while at 35° C. there was no appreciable difference whether they were in the air or in nitrogen. The tempera-

ture of the bath in the present experiments was 25.4 and 25.5° C. It seems improbable that the difference in behavior in the two sets of experiments could be due to a difference in temperature of only 4.5° C. Richards' experiments were conducted with material that had been sent from Tucson to New York and consequently it was not fresh, but the writer's material was taken from the plants only as it was to be used. In one of Richards' tables he gives the time as April 1–3, about two and a half months later in the season than the experiments here reported. At Tucson these months are very important as far as plant activity is concerned. In early January nearly every plant is dormant, while in late March everything is in full growth. Whether these differences in condition of the plants are significant or not is very hard to state. They may, however, account for the very great differences in results obtained. Richards continued his experiments for only a few hours. In the two experiments here reported the plants were exposed to nitrogen for 96 and 138 hours.

Experiment 36 and its control are illustrated by text figure 7. About 15 joints weighing 250 grams were used in this experiment. No determination of CO_2 after nitrogen was introduced was made until the following day because it had been found that several hours were required completely to remove oxygen from the respiration chamber. It will be noticed that for at least 40 hours after nitrogen was introduced the respiration was higher than before its introduction. During the following day the rate fell below the initial as well as below the control, which in this experiment remained fairly constant. At the end of 138 hours of exposure to the nitrogen atmosphere the respiration was still 54 percent of the original. This shows a remarkable ability to respire anaerobically. At the completion of the experiment only a few joints showed a few small grayish-green spots. Two weeks later those in soil were all in healthy condition with one exception. Those on the laboratory table showed slight browning in one specimen. These plants were thus injured only to a very slight degree.

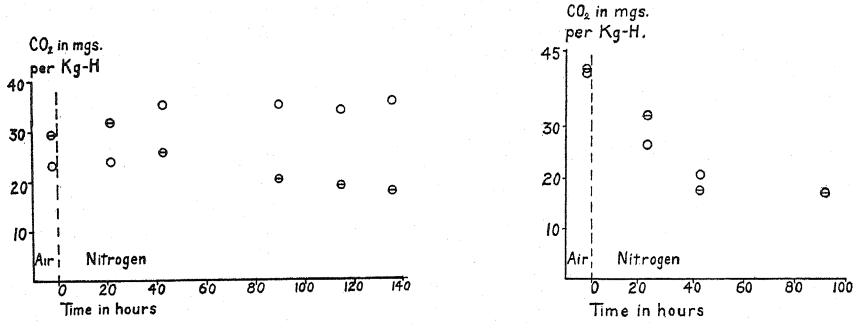
In the second experiment, which lasted only 96 hours, the rate fell quite rapidly in the control plants as well as in the experimental plants. The experimental plants were considerably injured at the end of the experiment and after 3 weeks in the soil they were all dead. The plants evidently were not in as good condition as in experiment 36, which was started 9 days later with material from the same plant. In experiment 36 all but two or three joints were plump and large in diameter, whereas in the first experiment most joints were slender. The slender joints were more injured in both experiments than the plump ones. The writer does not know whether the slender ones were younger than the plump ones or not.

Opuntia engelmannii

Opuntia engelmannii is one of the flat-jointed cacti with joints not over three-quarters of an inch thick, which are so strongly attached to the rest of

the plant that removal necessitates cutting, but of course the cut surface is very small. The material for the two experiments in which this species was used was obtained from two individuals, one for each experiment. The joints were the youngest ones on a branch.

Text figure 8 represents experiment 37 and its control. This experiment was conducted at the same time as experiment 36 so that the experimental conditions were identical. Of the whole group of experiments it is the most astonishing because at no time during the 138 hours that the plants were in nitrogen was the CO_2 production less than when they were in air at the be-



TEXT FIGS. 8 AND 9. FIG. 8 (left). Respiration of *Opuntia engelmannii* joints. Four joints were used in each jar. FIG. 9 (right). Respiration of *Echinocereus fendleri* plants. Five plants were used in each respiration chamber.

ginning of the experiment. It will be noted that though the CO_2 production decreased in the control day by day the experimental plants maintained practically the same rate from the second to the sixth day. At the end of the experiment and several weeks later no difference could be detected either externally or internally between those plants that had been in nitrogen and those that had been in air.

In the other experiment there was a fall in respiration below the initial after two days but the decrease was not as great as that in the control. Here also the plants were in perfect condition several weeks after they were taken out of the respiration chamber.

Here is a plant which is relatively thin, has an abundance of stomata, and should be abundantly supplied with air, and which one would expect to suffer quickly from lack of oxygen, yet it respires faster in absence of oxygen than in its presence, without injury to the plant tissue from the products of this incomplete respiratory process.

ECHINOCEREUS FENDLERI

Echinocereus fendleri is a cactus that grows in clusters of a dozen or more heads, which all come from one root by budding near the soil level. The plants are two to three inches in diameter and may grow as tall as ten inches.

For the experiments single plants were used either without buds or with very small buds. These plants were therefore rather young.

Two experiments, each with a control, were performed with this species. In one experiment air was reintroduced after 72 hours in nitrogen. The rate of CO_2 production increased gradually during the 3 days that the plants were in air, until the time nitrogen was reintroduced, when the respiration was 130 percent higher than it was just preceding the air introduction. When nitrogen was reintroduced the CO_2 production fell very abruptly to about the level at which it had been 4 days before when also in nitrogen. At the end of the experiment, 190 hours after the first introduction of nitrogen, the experimental plants respired as much as the control plants. At the beginning of the experiment they respired somewhat more. At the end of the experiment the plants that had been in the nitrogen had a few small yellow spots on the surface, but sectioning showed that these were only in the surface, where chlorophyll had been injured. The interior was apparently normal.

In the second experiment with *Echinocereus fendleri,* which is illustrated by text figure 9, 5 plants were used in each container. As will be noticed from the graph the experimental and control plants respired practically the same at the beginning of the experiment and at the end they were identical. In between, the control was higher one day and the experimental plants the next day. If a curve were drawn through the points the two would be very nearly the same. When the plants were taken out of the jar at the completion of the experiment one showed some spots that were slightly paler green than normal, but the others were not different in appearance from the control plants.

From these two experiments it is thus evident that *Echinocereus fendleri* is able to respire in the absence of air as readily as in air, without any or with very slight superficial injury. No injury deeper than a few millimeters was ever noticed.

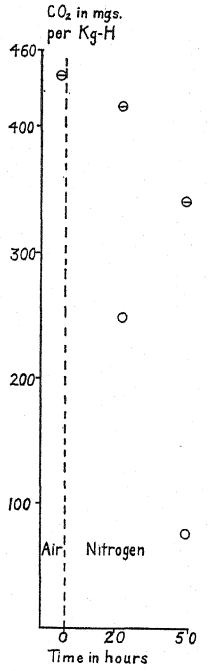
Leaves from Thin-leaved Plants

To determine whether other desert plants than cacti would behave in the same way as the plants investigated, the respiration of the leaves and young shoots of two perennials which had developed during December was also measured. The species were *Verbena ciliata* and *Encelia farinosa,* the leaves of which are of medium thickness, rather hairy in both plants.

The experiment with leaves of *Encelia farinosa* (text fig. 10) shows that 50 hours after nitrogen was introduced the respiration was only 17 percent of what it had been before. The leaves in air still respired 77 percent as much as they did at the beginning. The leaves of *Verbena ciliata* showed even a greater difference between those in nitrogen and those in air. These experiments on leaves leave no doubt as to the difference between the succulent cacti and non-fleshy plant structures. At the end of the experiment the leaves which had been in nitrogen were turning black and dried much sooner than the control leaves, when left on the laboratory table. These leaves were in to-

tal absence of oxygen not more than 41 hours at the most, as the gas passing through the bottle showed the presence of oxygen as late as 41 hours before the experiment was ended. In this experiment as in others the presence of oxygen was detected by passing the gas through a tube containing phosphorus. The presence of oxygen was readily detected by the formation of white phosphorus pentoxid.

These experiments show very conclusively that the succulent cactus plants are capable of existing in an atmosphere free of oxygen for several days with-



TEXT FIG. 10. Respiration of leaves and young shoots from *Encelia farinosa* plants. The leaves were not over two months old and many much younger.

out any injury beyond a few millimeters at the surface, and that most of the plants are not injured in the least. To this statement must be made the exception of two small plants of *Neomammillaria microcarpa* and the slender joints of *Opuntia versicolor*. In nitrogen they respired either more than the control, as in *Opuntia engelmannii*, or about the same as the control. From these experiments it is of course impossible to make any statement concerning the type of respiration which takes place under normal conditions. However, when one compares the injury produced in the thin leaves, caused by a very short period of anaerobiosis, with that in the cacti produced during a much longer period of oxygen absence, one is forced to believe that the interior of the cacti is accustomed to the products of anaerobic respiration. Spoehr (3)

has shown that in air *Opuntia versicolor* produces some alcohol, though of course much more alcohol is formed in the absence of oxygen. Further experiments must be conducted to test the assumption that anaerobic respiration takes place normally in the interior of cacti.

Summary

1. It has been shown that the cacti *Carnegiea gigantea, Ferocactus wislizeni, Neomammillaria microcarpa, Echinocereus fendleri, Opuntia engelmanii,* and *O. versicolor* are capable of producing as much or nearly as much CO_2 in absence of oxygen as they do in air. This is not true of the thin leaves of the desert perennials *Encelia farinosa* and *Verbena ciliata.*

2. When kept under anaerobic conditions for several days most of the cactus plants were not injured. Some were injured slightly in the surface layers, and one lot of *Opuntia versicolor* and two small specimens of *Neomammillaria microcarpa* were so badly injured that they later died.

3. Like tomato fruits, cacti showed a large increase in CO_2 production when air was introduced after the plants had been in nitrogen for some time.

The writer takes this opportunity to express his appreciation of the facilities extended him by the Carnegie Institution of Washington. He wishes especially to thank Dr. Shreve, the Director of the Desert Laboratory at Tucson, Arizona, for his thoughtfulness in supplying apparatus and material.

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ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, ATLANTIC CITY, N. J., DECEMBER 28–30, 1932 *

Dormancy in Seeds of Basswood, Tilia americana L. *J. Nelson Spaeth, Cornell University, Ithaca, N. Y.*—Various lots of basswood fruit and extracted seeds with germinative capacities between 70 and 95 percent gave no germination within 24 months in the greenhouse. Very little germination occurred in similar lots placed in moist storage at various refrigeration temperatures or in lots transferred to the greenhouse at 30-day intervals. No moisture reached the kernels of many seeds placed under moist conditions for 24 months. Seeds frozen in liquid air or in carbon dioxid snow and ether failed to germinate in 24 months. Extracted seeds treated 12 to 20 minutes with concentrated sulphuric acid gave 49 to 63 percent germination in four months when stratified at 2° to 5° C. Naked kernels in germinators at room temperature failed to germinate promptly. Under similar conditions naked embryos or embryos freed at the tip by dissecting away a small portion of the endosperm, exhibited geotropic curvature within 24 hours and at least one milimeter elongation of the hypocotyl within 48 hours. Increasing the oxygen pressure within a germinator induced germination of naked kernels with nucellar membrane intact. Removal or multiple perforation of the nucellar membrane enabled naked kernels to germinate without increased oxygen pressure. Apparently dormancy of *Tilia* seeds is due in part to the effect of the nucellar membrane on oxygen intake and in part to impermeability of the testa to moisture.

The Effect of Removing the Glumes on the Germination of Seeds of Poa compressa. *Alice M. Andersen, U. S. Department of Agriculture, Washington, D. C.*—Some seeds require light for germination. Seeds of Canada bluegrass (*Poa compressa*) have been generally classed in this group. To germinate these seeds, they are sown in Petri dishes on filter paper moistened with N/50 potassium nitrate solution and placed daily, in the light at 30° C. for 6 hours and in the dark at 20° C. for 18 hours during a period of 28 days. In a previous experiment on the germination of seeds of *Poa compressa*, the writer found that dilute nitric acid could be substituted for potassium nitrate in moistening the filter paper, giving an equal per-

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centage of germination. In this experiment seeds, with glumes removed by rubbing between several layers of cheese cloth, sown on filter paper moistened with water, were compared with seeds with glumes intact sown on filter paper moistened in one case with N/50 potassium nitrate and in the other with water. In one series several different samples of seeds with glumes removed, sown in Petri dishes on filter paper moistened with water and placed daily in the light at 30° C. for 6 hours and in the dark at 20° C. for 18 hours through a period of 28 days, gave 90 to 95 percent germination, which is equal to that obtained with seeds having glumes intact and moistened with N/50 potassium nitrate solution. The seeds with glumes intact and moistened with water gave 60 to 70 percent germination. Another series was conducted in the same manner except that the seeds received no light. Seeds with glumes removed gave about 60 percent germination, while seeds with glumes intact and moistened with N/50 potassium nitrate solution gave 80 to 85 percent germination. The seeds with glumes intact and moistened with water gave 20 to 30 percent germination.

The Influence of the Maturation Environment upon the Permeability of the Seed-coat of Barley. *W. H. Tharp, University of Wisconsin, Madison, Wis.*—Permeability studies were conducted with three hulless and two hulled varieties of barley. Uniform samples of each variety were grown (1) under a natural environment, which was relatively dry, and (2) under a field moist-cage where high soil moisture and high humidity were maintained during maturation. Harvestings were made from each variety, grown under the stated conditions, at 21 and 28 days following pollination and at maturity. The relative permeability of the seed-coats was determined in two ways: (1) By the rate of absorption from solutions of trichloracetic, nitric and sulfuric acids and iodine-potassium iodid, (2) By the electrical conductivity of seed-coat membranes in solutions of trichloracetic acid, iodine-potassium iodid, sodium chlorid and potassium iodid. A different rate of solute permeation was found, with both methods of determination, for each of the five varieties tested. Samples matured under the moist-cage were found more permeable than comparable samples of the same variety matured in the more arid environment. Likewise the samples harvested prematurely were found to be more permeable than similar fully matured kernels. These differences in relative permeability could not be correlated with any comparable variation in the anatomical structure of the seed-coat membranes. Microchemical tests failed to differentiate between the membranes from samples of barley exhibiting wide differences in rate of permeability.

Dormancy in Seeds of Symphoricarpos racemosus. *Florence Flemion, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—The seeds of *Symphoricarpos racemosus* are characterized by an extremely tough

seed coat. When seeds are stratified (mixed with moist peat moss) and kept at 1° or 5° C., about 5 to 10 percent germinate in 2½ years. When the seeds are treated with concentrated sulfuric acid for 45 to 60 minutes before stratification, about 40 percent germination is obtained after 7 months at 5° C. If, instead of being treated with sulfuric acid, seeds are stratified for four months at 25° or 30° C. before stratification at low temperature, 75 to 80 percent of seedlings results. Both the seed coat and the embryo are important in connection with the dormancy of these seeds. The effect of the tough seed coat can be overcome either by sulphuric acid treatment or by a period of high temperature stratification; while the dormancy of the embryo is broken by low temperature stratification.

Chemical and Physiological Studies of After-ripening of Seeds of Rhodotypos kerrioides. *Florence Flemion, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Seeds of *Rhodotypos kerrioides* require an after-ripening period at low temperature before germination takes place. When seeds are mixed in moist peat moss, better results are obtained if the seeds are stratified for one month at a higher temperature (25° C. or 30° C.) before the stratification at the low temperature (5° C.). If seeds are removed from the low temperature after they are partially after-ripened and kept at a high temperature and again placed at low temperature, they require a second after-ripening period just as long as if the seeds had not been partially after-ripened previously, indicating that at the higher temperature a secondary dormancy developed. Seeds that have been kept for several months in dry storage at room temperature produce a somewhat higher percentage of seedlings when subsequently subjected to low temperature treatment. Some changes apparently take place in dry storage which make the seeds more responsive to germinative conditions. A study of the behavior of excised embryos showed that both the embryo and the seed coat play a rôle in the dormancy of these seeds. Analyses of the seeds at intervals of two weeks during the after-ripening period show that the seeds increase in catalase, peroxidase, and lipase activity, and also increase in water absorption power, N soluble in 80 percent alcohol, titrable acid, and sucrose. The ether soluble fraction decreases as after-ripening progresses.

Dwarf Plants from Unafter-ripened Embryos of Seeds of Rhodotypos kerrioides. *Florence Flemion, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—The seeds of *Rhodotypos kerrioides* require an after-ripening period of several months at 5° C. but if the inner and outer coats of seeds not after-ripened are removed and the embryos thus excised are mixed with moist peat moss at 25° C. and subsequently transferred to soil, a small percentage of seedlings results. The seedlings thus produced however grow much more slowly than normal and the resulting plants have the appearance characteristic of dwarfs. In the experiments with seeds of the

1931 crop, the plants exhibited this abnormal growth for about 4 to 7 months, after which the terminal bud started to grow in a normal manner similar to the plants which developed from after-ripened embryos. It seems that in the case of unafter-ripened embryos some factor necessary for normal growth is lacking, and that this factor develops after several months in the dwarf seedlings without a low temperature period. Embryos from seeds which have been in dry storage for several months, produce more nearly normal seedlings. It is not known at this time whether this is due to changes which have taken place within the embryo or to the more favorable light conditions prevailing when these were planted (April to June). These phenomena are being investigated further.

The Daily Growth of Zea mays. *W. E. Loomis, Iowa State College, Ames, Iowa.*—Young corn plants grow in height only when the temperature is above a minimum of approximately 10° C. and the *growing point* is well supplied with water. Direct sunlight causes a marked water deficit in the above ground portions of the corn plant even though the roots are liberally supplied with moisture, and quickly stops elongation. Growth is not checked by light when the light intensity and humidity relations are such that the growing point is able to obtain a normal supply of water. The most rapid growth rates in well watered corn were obtained just before sundown or on warm, cloudy days.

Toxicity of Normal and Pathological Blood for Living Seedlings. *Dr. David I. Macht, Hynson, Westcott & Dunning, Inc., Baltimore, Md.*—Young seedlings of *Lupinus albus* are placed in physiological solutions, and the growth of the roots in the dark and at constant temperature is carefully measured and compared with that of another series of seedlings from the same crop, suspended in similar saline plus one percent of blood serum. Normal sera from fourteen different species of animals, including man, were studied. These produced a mild inhibition of growth. There is very little difference in toxicity for plants between normal blood sera of different species of animals. Blood of human beings under certain physiological and pathological conditions, however, exhibits greater toxicity than normal blood. Thus, in menstrual blood there is a toxin producing marked inhibition of growth and other phytotoxic phenomena. Again, in primary or pernicious anemia, another toxic substance is found, which cannot be detected by zoöpharmacological methods and which is not present in other forms of anemia, such as secondary anemia, leukemia, anemia due to cancer, etc. Other toxins demonstrable by phytopharmacological methods are those of pemphigus and leprosy. Blood corpuscles and serum in carbon monoxide poisoning are very toxic for plants. Acute bacterial infections produce no phytotoxic substances. Phytopharmacological studies throw light on the etiology of certain diseases, are helpful in differential diagnosis and are useful in following the progress of therapeutic procedures.

Neophyosis or Rejuvenescence of Nucellar Bud Seedlings in Citrus. *Walter T. Swingle, U. S. Department of Agriculture, Washington, D. C.*—The embryo sac is frequently invaded by few-celled buds of nucellar tissue which in developing recapitulate very nearly the normal morphological structures of the true embryo-cotyledons, epicotyl, and intermediate leaves, hypocotyl, collar, tap and primary lateral roots. Recently originated ovary-sterile hybrids reproduce almost exactly the original F 1 seedling but old cultivated varieties long propagated vegetatively usually exhibit marked rejuvenescence and probably revert approximately to their original youthful state. This unexplained recapitulation of embryonic and juvenile morphology may be due to hormones secreted in the embryo sac apparatus that force into activity, in the nucellar bud cells, genes governing embryonal ontogeny. The accompanying neophyosis or rejuvenescent action is possibly a by-product of these powerful influences which in forcing cells of old, more or less senescent, tissue to produce juvenile structures may, at the same time, cause them to take on the physiological activities appropriate to young and vigorous plants.

The Decomposition of Chlorophyll by the Animal Body. *O. L. Inman and Paul Rothemund, Antioch College, Yellow Springs, Ohio.*—Contents of the stomachs of cows and sheep fed on ensilage and on grass were used to study the fate of chlorophyll in the animal body. It was found that even in the first stomach the complex molecule of chlorophyll underwent partial reduction, but that the seat of the major changes in the chlorophyll molecule occurred in the third stomach, from which four porphyrins were isolated. The subsequent rôle of these decomposition products in the organism is being studied. Preliminary work on the feeding of rats indicates that one at least of these reduction products of chlorophyll may have some definite physiological relationship to the blood pigment hemoglobin, the basic structure of which is similar to that of chlorophyll.

Fiber Abnormalities and Pressure Variations within the Boll in Gossypium. *Wanda K. Farr, U. S. Department of Agriculiure, Washington, D. C., and Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—*G. barbadense* L. (Pima) and *G. hirsutum* L. varieties Super Seven and Acala show increasing numbers of fiber abnormalities in the order named and in the ratios of 15 : 90 : 170. Very few abnormalities occur before the twentieth day from the time of fertilization in any one of the varieties. Measurements of pressure within the boll during the entire period of development indicate that it is relatively small in Pima, moderate in Super Seven, and large in Acala. The pressure increases from the twentieth day until near the time of dehiscence. These observations suggest the possibility that pressure may be one of the important factors in the formation of fiber abnormalities.

Effect of Various Chemicals on the Respiratory Rate and Sugar Content of Potato Tubers. *Lawrence P. Miller, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—When whole tubers are subjected to the vapors of such chemicals as ethylene chlorhydrin, ethylene bromohydrin, ethyl mercaptan, hydrogen sulfid, ethyl disulfid, and acetaldehyde, large increases in the respiratory rate take place. (Ethyl alcohol shows an exceptional behavior in causing a decrease in respiratory activity.) These increases occur very soon after the beginning of the treatment (24-hr. treatment period), and reach their maximum the second or third day after the treatment, after which the respiratory rate of the treated tubers gradually falls to a value approaching that of the untreated samples. Although many of these treatments result in increases in the sucrose content of the tubers and respiratory responses have often been attributed to sugar changes, the results do not indicate that this increased respiratory activity is caused by the sugar increases. This is shown by the ethylene chlorhydrin treatments in which the largest respiratory increase takes place very definitely before the sugar increase and there is in fact a drop in sugar content at the period of greatest respiratory activity. The sugar content subsequently rises while the respiratory rate is falling.

The Behavior of the Nucleus of the Yeast S. cerevisiae Hansen During Cell Division (Budding). *Oscar W. Richards, Yale University.*—Further knowledge of the nuclear behavior of yeast is necessary for the interpretation of studies in progress on the growth of the individual yeast cell at various times during the cycle of population growth and on the effect of X-radiation on the growth of yeast populations. Mitotic division of this yeast has been reported by Fuhrman, Swellingreble, and Kater. This view is opposed by Wager, Wager and Peniston, Guilliermond and other investigators. Re-examination of this problem by staining (including Feulgen), observation with the Ultropak lens system and the ultraviolet microscope fails to show mitotic division in this species.

A Comparison of Osmotic Values of Supplying and Receiving Tissues and its Bearing on the Münch Hypothesis of the Translocation Mechanism. *O. F. Curtis and H. T. Scofield, Cornell University, Ithaca, N. Y.*—Münch has proposed a hypothesis to account for the mechanism of transport of solutes in plants. This hypothesis demands a pressure gradient leading from the supplying cells to the receiving cells. This pressure gradient is supposed to result from an osmotic gradient and to cause a unidirectional mass flow of cell contents. Osmotic concentrations of several types of supplying and receiving tissues have been determined by direct freezing-point measurements of the tissues concerned, by measurements of the freezing points of extracted saps and by plasmolytic measurements with sucrose solutions. Such measurements were made with the following types of materials: potato tubers and the sprouts developing from them; cotyledons

of beans and squash and growing regions of the dependent seedlings; parent leaves of *Bryophyllum* and *Sedum* and the plantlets developing from them; storage scales of onions and the young leaves as well as root tips drawing their supplies from them. For each type of material tested, the osmotic gradient leads from the receiving tissue to the supplying tissue. Although this does not conclusively demonstrate a turgor gradient in the same direction, it does strongly indicate such a gradient. General observations of great flaccidity in storage organs, which are supplying more turgid growing tissues, support the evidence from quantitative determinations of osmotic concentration that turgor gradients frequently, if not consistently, lead from the receiving cells to the supplying cells. This evidence clearly tends to refute the hypothesis of Münch which demands a pressure gradient in the reverse direction, that is from the supplying cells to the receiving cells.

Observations on the Effect of pH Value on Oxidase Activity. *W. V. Cruess, R. N. Jeffrey, and H. M. Pancoast, University of California, Berkeley, California.*—Oxidase activity as measured by the rate of absorption of oxygen by the juices expressed from peaches, apricots, olives, apples, avocadoes, asparagus and spinach was greatly affected by pH value. Various pH values were secured by acidification with citric acid and by addition of NaOH. Absorption of oxygen practically ceased at pH values below 2.5. At pH values above pH 4 absorption was more rapid than at pH values below 4. At pH values above 7 (alkaline range) absorption of oxygen was naturally greatly stimulated, although much of the oxidation occurring in the alkaline range was non-enzymic since absorption of oxygen by the alkaline juice was appreciable in the absence of oxidase. Catechol was added to the juices as a substrate for enzymic oxidation and was found to be more satisfactory than several other oxidizable compounds with which it was compared. Absorption was measured by means of a gasometric apparatus in which the volume change was determined volumetrically rather than manometrically. CO_2 formed during the experiment was absorbed in NaOH in order that it would not affect the volumetric readings.

The Electrokinetic Potential of Latex as an Indicator of Plant Relationship. *Lawrence S. Moyer, University of Pennsylvania, Philadelphia, Pa.*—Measurements of the isoelectric points of latex particles were made by suspending the latex in buffers and studying its electrophoretic mobility in a modified Northrup-Kunitz electrophoresis apparatus. The curves of mobility (or of electrokinetic potential) plotted against pH proved to be quite constant for each species investigated. Over twenty species of the genus *Euphorbia* were so studied. It was found that their curves and isoelectric points fall into groups of corresponding shapes. These groups agree with the taxonomic relationships already established for the genus. Further support is given to the evidence that the surface of the latex particle is at least in part coated with protein.

The Distribution of Cytisus scoparius in Virginia with Special Reference to Soil Reaction. *Thomas W. Turner, Hampton Institute, Hampton, Va.*—*Cytisus scoparius* or Scotch Broom is found growing naturally in small patches or along roadsides in four or five different regions of the State: around Yorktown and vicinity, Williamsburg and vicinity, Amelia, Hampden-Sydney and Lynchburg. The time and method of its introduction into this section is not definitely known, though it is generally ascribed to the period of the Revolutionary campaigns about Yorktown. A study of the pH values of the soils in which it is found growing shows a rather narrow range, lying between the averages 5.9 and 6.7. Preliminary germination experiments with five selected soil types showed leaf mold the most favorable. Several factors must be considered in explaining the slow natural distribution of Scotch Broom in this area, namely, its narrow pH range, frequent tillage driving it from the open places and probably certain soil factors limiting seed germination.

Some Observations on pH Values of Plant Juice and Sap. *Arthur Willis Barton, Kansas State College, Hays, Kansas.*—This investigation is being carried on for the purpose of discovering whether there are such great variations in the hydrogen-ion concentration of the juice of plants as the literature indicates; whether the sap obtained in the early spring when it is " flowing " varies as compared with sap or juice expressed from the plant tissue; and whether plants that have been injured or become infested with fungi and other parasitic organisms have had any change in acidity or alkalinity of the liquid that may be obtained from them by appropriate means. The determinations of hydrogen-ion concentrations have been made with an apparatus using a quinhydrone electrode, also, where possible, with a colorimeter scale. The juice, when not flowing, has been obtained by grinding in a food chopper and pressing the liquid from the mash thus obtained. Most of the data so far obtained indicate that the liquid obtained from plants is slightly acid; and that abnormal conditions, such as smut-infested corn, produces a slight reduction in the amount of acidity, the juice obtained from the smut being nearly alkaline.

Relation of Growth Cycle to Toxicity of Wound Dressings. *J. S. Cooley and J. H. Crenshaw, U. S. Department of Agriculture, Washington, D. C.*—In connection with work on wound dressings on apple trees at Hood River, Oregon, it was observed that there was considerable variation in toxicity of the same dressing when applied to fresh cuts. Comparable wounds were made periodically every month during the year and treated immediately with 3 different types of dressing, viz: Bordeaux paste composed of dry Bordeaux mixed with water; Bordeaux mixed with oil to the consistency of paste; white lead with linseed oil and a mixture of 4 parts copper soap, 7 parts rosin, and 3 parts fish oil. It was noted that killing was very pronounced in the fall, even by very mild dressings, and that there was

either no killing or very slight injury from the same dressings when applied on wounds made in the spring. The close relationship of resistance to toxic dressings and the life activity of the plants is attested by the similarity of the curves of wound callus formation and resistance and susceptibility to injury by wound dressings. Further evidence of some intimate connection between toxicity and vital phenomena is also supplied by the fact that the curves for positive perennial canker (*Gloeosporium perennans*) infection as obtained by Shear and Cooley, and the curve for toxicity to wound dressings are also very similar.

Carbon Dioxid Storage. III. The Influence of Carbon Dioxid on the Oxygen Uptake by Fruits and Vegetables. *Norwood C. Thornton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Carbon dioxid as one of the end products of respiration in plants might be expected, upon accumulation, to exert an inhibiting influence on the respiratory processes of the tissues. The present experiments show, however, that in the presence of 20 to 70 percent CO_2 the rate of oxygen uptake by the tissues is different for different tissues and may show increases, decreases, or no effect depending upon the tissue studied. The percentage increases or decreases in the oxygen uptake by various tissues stored in 55 to 65 percent CO_2 with 20 percent O_2 were as follows: Increases by potato (dormant) 83 percent, (non-dormant) 239 percent, (sprouting) 117 percent; onion bulbs 89 percent; tulip bulbs 11 percent; beet roots 95 percent. Decreases by asparagus shoots 33 percent; banana fruit 17 percent; strawberry fruit 17 percent; lima bean (green) in pod 15 percent; shelled 34 percent. Varying the CO_2 content from 8 to 72 percent did not definitely influence the rate of oxygen uptake by carrot roots. The period of storage in the gas is important. The results described above for potatoes were obtained during a period of 41 to 144 hours storage; for periods of less than 20 hours decreases rather than increases were obtained. The storage periods in hours for the other tissues mentioned above were as follows; onion bulbs 90; tulip bulbs 68; beet roots 64 to 92; asparagus shoots 4 to 24; banana fruit 6 to 37; strawberry fruit 4 to 38; lima bean 17 to 24; and carrot roots 21 to 115.

Carbon Dioxid Storage. IV. The Influence of Carbon Dioxid on the Acidity of Fruits and Vegetables. *Norwood C. Thornton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Since CO_2 has acidic properties it might be expected that in the presence of a sufficient concentration of CO_2 plant tissues would become more acid. These experiments show, however, that storage in CO_2 gas causes the juices of plant tissue to become more alkaline. The reduction in acidity of the tissue increases with increasing concentration of CO_2 and with the prolongation of the storage period. With some tissues the change is very rapid starting within a few hours in storage in 20 percent O_2 and 30 to 60 percent CO_2. After storage in such a gas mixture the pH increase, over the control, in the extracted

juice of various tissues was approximately: potato 0.5 to 0.7, carrot 0.5 to 0.8, asparagus 0.3 to 0.6, beet 0.3 to 0.8, lima bean 0.2 to 0.3, onion bulb 0.6, apple fruit 0.3, tulip bulb 0.2 to 0.3, banana pulp 0.2 to 0.6, and strawberry fruit 0.17. After removal of dissolved CO_2 from the extracted juice of potato a greater change in alkalinity, than shown above, was found. This change in the alkaline direction in the presence of CO_2 is dependent upon the presence of oxygen. In the absence of oxygen the change in pH is not in the alkaline direction, but in the direction of increased acidity.

Nitrogen Fixation by Myxophyceae. *Joseph J. Copeland, College of the City of New York, New York City, N. Y.*—The general status of nitrogen fixation is considered briefly. Evidence on the fixation of molecular nitrogen by blue-green algae (Myxophyceae) is presented. This data was obtained by culturing various strains in nitrogen deficient media; it consists of measurements of the ability or inability to grow normally in the absence of combined nitrogen, data on weight increment in such cultures, and direct measurements of the increment of total nitrogen in them. The following conclusions are reached: (1). Nitrogen fixation is carried on by certain Myxophyceae, including strains of *Oscillatoria princeps*, *Oscillatoria formosa*, *Spirulina labyrinthiformis* and *Phormidium laminosum;* other Myxophyceae lack this ability. (2). In this study the nitrogen fixers found were entirely strains from thermal sources; some thermal strains do not seem to possess the ability to assimilate free nitrogen. (3). In this study no nitrogen fixers found had been secured from waters that contained much combined nitrogen; the nitrogen fixers found were secured from nitrogen deficient warm springs. (4). The appearance of blue-green algae grown in nitrogen deficient media gives excellent circumstantial evidence of the ability or inability of the plant to fix nitrogen. (5). Evidence is given for the existence of physiological strains of *Oscillatoria princeps* and *O. formosa*, differing in their abilities to assimilate molecular nitrogen. It is suggested that physiological specialization may occur in other blue-green algae.

Soil Aeration in Relation to Plant Metabolism. *W. F. Loehwing, University of Iowa, Iowa City, Iowa.*—Sand cultures of sunflowers supplied with neutral Knop's solution were aspirated with fifty liters of air daily. Aerated plants exhibited accelerated growth and greater total stature, larger tops and roots but were lower in percentage ash, calcium, phosphorus, soluble sugars, hydrolyzable polysaccharides and total nitrogen, though they were higher in absolute amounts of the above constituents. Aerated tops were higher and roots lower in percentage potassium though controls contained smaller total amounts. Top-root ratios of aerated plants were much lower. Expressed sap of aerated plants was higher in total solids, lower in ash, higher in sugars, lower in total non-nitrate nitrogen, higher in oxidation potential, lower in hydrion concentration. Sap of aerated roots was less efficiently buffered against acid and alkali in both young

and mature plants. Tops, however, were more poorly buffered against acid than controls. Specific conductivity of root sap was higher for aerated plants but was lower for the sap of young aerated plants though it finally reached the same level as controls in mature plants. The cryohydric point of control sap was generally lower than that of aerated cultures. The above effects appear definitely ascribable to the accelerated growth, altered habit and larger bulk of the root system of aerated plants.

Assimilation of Carbon Dioxid by Apple Leaves under Natural Conditions. *A. J. Heinicke, Cornell University, Ithaca, N. Y.*—Marked fluctuations in the hourly and daily rate of photosynthesis of apple leaves under natural conditions are indicated by the difference in CO_2 content of "normal" air and of air which has passed over attached leaves enclosed in special assimilation chambers. The highest rate in over 1000 determinations during 1 to 5 hour periods was 35 mg. CO_2 per hour per hundred Cm^2; the most common rate between 5 to 10 mg. In order to obtain relatively high values, the flow of "normal" air on clear days should be about 1 liter per hour for each Cm^2 of leaf surface. Occasionally the CO_2 respired by the leaves during daylight hours exceeds assimilation, but this occurred in not over 5 percent of the cases. The rate of respiration of leaves in the dark may be as high as 8 mg. CO_2 per hour per 100 Cm^2 of leaf surface, but it is usually less than 1 mg. The yields during 5-hour periods on bright days may be 3 to 5 times as much as on warm, cloudy days. As a rule more CO_2 is absorbed in the morning than in the afternoon, but high values may occur a few hours before sunset. Under the same set of external conditions the assimilation of carbon dioxid per unit leaf area of plants of the same variety and age may vary as much as 500 percent. The rate is clearly influenced by such internal conditions as water supply, nitrogen supply, the intensity of green color of the leaf, and the general state of vigor of the plant. Under certain conditions, spraying and ringing, which do not visibly affect the leaf surface, may greatly reduce photosynthesis.

Effects of Illuminating Gas on Young Oak Trees. *Carl G. Deuber, Yale University, New Haven, Conn.*—The roots, dormant buds and entire potted trees of red and black oak trees were subjected to atmospheres of a "mixed" illuminating gas or high concentrations of this gas and air. With dormant trees, each of the above methods of subjecting a part of the tree or the entire tree to gas resulted in hastening the opening of the leaf buds with short exposures to certain volumes of gas. Longer exposure periods and larger volumes of gas inhibited the development of the buds or killed the trees. Hypertrophy of the lenticels of the roots occurred when the roots were exposed to gas. Subjection of the roots of trees in foliage to gas resulted in a temporary or permanent wilting of the leaves, epinastic growth of the petioles and a partial or complete loss of leaf pigments.

The Effect on Plants of Passing Illuminating Gas through the Soil. *A. E. Hitchcock, William Crocker, and P. W. Zimmerman, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Root systems of potted plants were subjected (1) directly to flowing illuminating gas, (2) to illuminating gas after first passing through water, sodium hydroxid, soil, and moss peat, (3) to soil containing residues from a previous passage of illuminating gas, (4) to gassed soil after leaching with water, (5) to soil which was gassed and aerated, and (6) to soil containing a liquid residue condensed by low temperature fractionation from illuminating gas. Injury to the plant was prevented or lessened when illuminating gas was first passed through water, sodium hydroxide, soil, or moss peat, depending on the total amount of gas and the kind of plant used. Residual products remaining after passing illuminating gas through soil caused injury or death, although no part of the plants had been subjected to flowing gas. Gassed soil nearest the inlet was more toxic than that near the outlet. Moderate leaching of gassed soil reduced only slightly the injurious effects on the plants. Aeration of gassed soil lessened but did not always prevent injurious effects on the plant. Some of the effects on the aerial parts as a result of gassing the root systems in soil were epinasty, leaf-fall, change in color, proliferation, wilting, drying, necrosis, forcing of latent shoot buds, and stimulation and initiation of root primordia along the stem. Root injury in gassed soil was evidenced by retarded growth, proliferation of main roots and killing. Relative toxicity of gassed soils was also determined by measuring cyanogen, phenols, and heavy hydrocarbons.

Initiation and Stimulation of Roots from Exposure to Carbon Monoxid Gas. *P. W. Zimmerman, William Crocker, and A. E. Hitchcock, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Potted plants of 80 species were exposed in Wardian cases to carbon monoxid. This gas induced definite rooting responses in 30 species of herbaceous and woody types. The following variations in response were noted: (1) initiation of adventitious roots from young tissues on the stems or leaves, (2) stimulation of preexisting root primordia on the stems, (3) modification of soil roots in response to gravity, and (4) various combinations of these three types of response. The gas was effective over a wide range of concentrations from 0.05 percent to 50 percent. The time of exposure necessary to induce the response varied with the species from two days for the tomato to twenty days for hydrangea. Carbon monoxid induced root initiation in young stem tissue of 11 species; caused stimulation of preexisting root primordia in 20 species; caused special nodal rooting in 8 species; and induced roots to form on leaves of 3 species. *Hydrangea macrophylla*, *Nicotiana tabacum*, and *Impatiens balsamina* responded by producing roots from a short zone on the stem representing the region of elongation when the treatment began. Tobacco cuttings from tips of treated plants grew large root systems in five days. Control cuttings required 15 days to produce comparable roots.

Carbon monoxid is specific for inducing root formation, no shoot primordia having formed. Three other gases, acetylene, propylene, and butylene, have been found effective for inducing root primordia to form somewhat as reported for carbon monoxid.

The Effect of Carbon Monoxid on Plants. *P. W. Zimmerman, William Crocker, and A. E. Hitchcock, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Ninety-six species of plants have been exposed in Wardian cases to various concentrations of carbon monoxid gas. The plants varied widely in their susceptibility and types of responses, the most important of which were as follows: (1) epinastic growth of leaves, (2) modification in rate of aging of leaves, (3) abscission of leaves, flower buds, and fruits, (4) abnormal development of lenticular tissue, (5) modification in rate of growth, and (6) modification in size of leaves produced while the plants were in the gas. Thirty-six species showed epinasty of leaves; the majority of the species tested showed an increase in the rate of aging and early abscission of old leaves. Three species showed abscission of the young leaves earlier than the old ones. Growth was retarded in varying degrees but not entirely stopped. New organs produced while the plants were in gas were usually abnormal. Leaves, for example, became progressively smaller as the treatment was continued. *Mimosa pudica* in one percent carbon monoxid became anaesthetized and became insensitive to external stimuli. Motion pictures show that the leaves lose their correlation and move rapidly in various directions.

The Effect of Ether and Chloroform upon the Rate of Photosynthesis in Detached Leaves of Acer negundo. *Raymond H. Wallace, Conn. Agricultural College, Storrs, Conn.*—A series of quantitative experiments with ether and chloroform has shown that it is possible to stop the process of photosynthesis only with lethal concentrations of these vapors. The experiments were run during the months of June, July and August. All tests were made on detached leaves in hermetically sealed containers immersed in a constant temperature bath at 25° C. Inlet and outlet tubes on the sealed chambers enabled one to make up any desired combination of gases and analyze these atmospheres at any time. The rates of photosynthesis were determined by gas analysis of the enclosed atmospheres after given exposures to light. Figures and descriptions of the apparatus are given. The concentrations of ether tested ranged from 2 percent ether vapor and 98 percent air to 35 percent ether vapor and 65 percent air. Even in the highest concentration the process did not cease at once, but only gradually along with leaf discoloration and other evidences of death from the ether treatment. Concentrations causing no apparent injury to the leaves likewise had no apparent retarding effect on the process. On the contrary there were numerous cases in which the application of sublethal

concentrations of ether vapor increased markedly the rate of photosynthesis. This increase in the rate can probably be attributed directly to the increase in stomatal opening on exposure to ether. The stomata of many plants will open when placed in water solutions of ether. A series of chloroform concentrations from ½ percent to 5 percent has failed to show either a definite increase or decrease in the rate of photosynthesis. It has likewise been impossible to obtain opening of stomata on treatment with chloroform vapor. Tables and curves for the various tests are given.

Eliminating the Use of Calcium Carbonate in Preparing Plant Tissue for Analysis. *F. E. Denny, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.*—The addition of calcium carbonate in the preparation of plant tissue for analysis has been assumed to be necessary in order to neutralize plant acids and to prevent the hydrolysis of sucrose. Since the excess carbonate in the plant residues interferes with analyses for various constituents, a satisfactory procedure omitting calcium carbonate would be desirable. In determining the sucrose content of the tissues of 13 different species of plants it was found that similar values were obtained whether or not calcium carbonate was added to the boiling alcohol into which the sample of tissue was dropped in preparing the tissue for analysis. It was found that calcium carbonate did not neutralize the acidity of juices of acid fruits in alcoholic solution, the pH values being displaced only slightly in the alkaline direction. The hydrolysis of sucrose by the organic acids, oxalic, citric, tartaric, and malic, in 70 percent alcoholic solution was measured at different pH values and with different periods of heating. With one hour's heating in boiling 70 percent alcohol, no hydrolysis occurred at pH 5.0, with any of the acids, and even at pH 4.0 inversion by citric, malic, and tartaric was slight, only oxalic being effective under these conditions. At pH 3.0 measurable hydrolysis was found but by suitably adjusting the temperature of the alcohol and the duration of contact with alcohol at that temperature, conditions for the preparation of tissues for analysis and for their extraction without inverting sucrose were found.

Weight of Plant Tissue Produced in Relation to the Efficiency of an Incandescent Filament Lamp. *John M. Arthur, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Buckwheat seedlings were grown for periods of 12 to 14 days with 500-watt lamps operating continuously at 10, 20, and 30 lumens-per-watt efficiency, respectively. Air temperature and humidity were held constant by standard air-conditioning machinery. The 10, 20, and 30 lumen-per-watt lamps at 33.5, 42.5, and 57.5 inches, respectively, above the soil gave the same foot-candle illumination as measured by the Weston Photronic cell. With the lamps arranged at these distances the dry and wet weights of plant tissue produced were also approximately the same. The illumination under each lamp at the soil level was approximately 205 foot candles. The total energy value at the

soil level under the 30 lumen-per-watt lamp as measured by three different types of thermopiles was found to be only 49 percent of that under the 10 lumen-per-watt lamp. The dry weight production of plant tissue follows closely the foot candle intensity as measured by a Weston Photronic cell with a maximum sensitivity in the yellow region and is not related to the total energy output of the lamps as measured by thermopiles. Energy in the infra-red region is therefore of no value in the production of dry weight when plants are grown under these conditions, at an air temperature of 25.5° C.

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